



ESCOLA UNIVERSITÁRIA VASCO DA GAMA
MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

FELINE LEISHMANIASIS: A REVIEW

Carla Sofia Alves Soares

Coimbra, Abril de 2014



ESCOLA UNIVERSITÁRIA VASCO DA GAMA
MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

FELINE LEISHMANIASIS: A REVIEW

Autor: Carla Sofia Alves Soares

Aluna do Mestrado Integrado em Medicina Veterinária

Orientador Interno: Professora Doutora Sofia Duarte

Escola Universitária Vasco da Gama

Co-Orientador: Dr. Sérgio Sousa

Escola Universitária Vasco da Gama

Coimbra, Abril de 2014

**Dissertação do Estágio Curricular
do Ciclo de Estudos Conducente ao
Grau de Mestre em Medicina Veterinária da EUVG**

Table of Contents

List of Figures	vi
List of Tables	vi
List of Abbreviations	vii
Title page	1
Abstract	2
Keywords	2
1. Introduction	3
2. The Parasite	5
3. Vectors Bioecology	7
4. Epidemiology of Feline Leishmaniasis	8
5. Pathogenesis and Lesions	13
6. Immunological Features of <i>Leishmania</i>	15
7. Diagnosis	18
8. Therapy and Prevention Strategies	21
9. Final Remarks	24
10. Conflict of Interest Statement	25
11 Acknowledgments	25
12. References	25
Annex	35

***“Cada sonho que se deixa para trás,
É um pedaço de Futuro que deixa de existir.”***

Steve Jobs

Para a Fiona, Mary e a todos Vocês que instigaram este meu caminho,
Que agora se concretiza! Foi decisivo crescer convosco!

Aos meus queridos Avós, pelo significado que trouxeram à minha infância...
E pelo vazio que deixam no meu Futuro...

Ao meu Pai, por demonstrar a assertividade, a racionalidade, a honestidade,
O sacrifício para ter o retorno...

À minha mãe, por me ter ensinado a ser criativa, a construir para ter, a nunca desistir,
E a trabalhar para atingir o objectivo.

Aos meus Mentores e Orientadores, pelo incentivo e por acreditaram em mim...
Perspetivaram-me caminhos Geniais de consolidação profissional e pessoal!
Foram uma inspiração, E continuarão a ser...

Aos meus estimados Amigos e a todos os momentos Bons que tive...
Mas, especialmente, a todos os momentos menos bons...
Por me terem aguçado o perfeccionismo e a perspicácia do que designo Viver!

List of Figures

Figure1. Suggested immune response of felines in *Leishmania* infection, according with immune mechanism in CaL

List of Tables

Table 1: Compilation of worldwide epidemiologic surveys of Feline Leishmaniasis due to *Leishmania infantum*

Table 2: Compilation of worldwide case reports of Feline Leishmaniasis due to *Leishmania infantum*

List of Abbreviations

ATP: Adenosine Triphosphate

CaL: Canine Leishmaniasis

CD4: T-helper cell

CD8: T-cytotoxic cell

CL: Cutaneous Leishmaniasis

CMI: Cell-Mediated Immunity

cPCR: Conventional Polymerase Chain Reaction

DAT: Direct Agglutination Test

DDT: Dichlorodiphenyltrichloroethane

DTH: Delayed-type Hypersensitivity Test

DNA: Deoxyribonucleic Acid

ELISA: Enzyme-Linked Immunosorbent Assay

FeL: Feline Leishmaniasis

FeLV: Feline Leukemia Virus

Fe-SOD: Iron Superoxide Dismutase

FIP: Feline Infectious Peritonitis

FIV: Feline Immunodeficiency Virus

WHO: World Health Organization

HIV: Human Immunodeficiency Virus

HuL: Human Leishmaniasis

IFI: Indirect Immunofluorescence

IFN- γ : Gamma Interferon

Ig: Immunoglobulin

IL: Interleukin

IM: Intramuscular

MCL: Muco-Cutaneous Leishmaniasis

MHC: Major Histocompatibility Complex

NO: Nitric Oxide

NK: Natural Killer cells

ODD: Obligatory Disease Declaration

P: Plasma cell

PNLVERAZ: *Programa Nacional de Luta e Vigilância Epidemiológica da Raiva Animal e Outras Zoonoses* (National Program for the Control and Epidemiological Surveillance of Animal Rabies and other Zoonoses)

PO: Per Os

qPCR: Real-time Polymerase Chain Reaction

R₀: basic reproduction number

RNA: Ribonucleic Acid

RFLP: Restriction Fragment Length Polymorphism

SC: Subcutaneous

SID: once daily dosing

TC: Cytotoxic Cell

TGF- β : Beta Transformation Growth Factor

Th1: Type-1 T-helper cell

Th2: Type-2 T-helper cell

TNF- α : Alpha Tumor Necrosis Factor

Treg: regulatory T-cell

VL: Visceral Leishmaniasis

WB: Western Blot

FELINE LEISHMANIASIS: A REVIEW ★

Carla Sofia Alves Soares¹, Sofia Cancela Duarte^{1,2}, Sérgio Ramalho Sousa^{1,3}

¹Department of Veterinary Medicine -Escola Universitária Vasco da Gama, Av. José R. Sousa Fernandes, 3020-210 Coimbra, Portugal: carlasoares.medvet@gmail.com

²Group of Health Surveillance, Center of Pharmaceutical Studies, University of Coimbra, Polo III, Azinhaga de Sta Comba, 3000-548 Coimbra, Portugal: sofiacanceladuarte@gmail.com
Faculdade de Farmácia da Universidade de Coimbra, Portugal

³CIISA, Faculty of Veterinary Medicine, University of Lisbon, Alto da Ajuda, Lisbon, Portugal: ramalhosousa@gmail.com

★ This dissertation was written according to the formatting rules of a review paper as required by the journal *Veterinary Parasitology* (Annex).

Abstract

According to the World Health Organization (WHO), Leishmaniasis' endemic areas have spread and the prevalence of the disease has increased, as well as the number of reported cases. Europe is one of the most affected continents concerning the risk of re-emergence of this zoonosis.

Feline Leishmaniasis (FeL) was for the first time described in Algeria, 1912. The significance of the cat as a reservoir of *Leishmania* and not simply an alternative host seems to be gaining ground, mainly because: i) cats can present increased seropositivity between serology analysis; ii) cats can be infected during some months and thus are available for sand flies; iii) cats transmit the *Leishmania* agent in a competent form.

Furthermore, cats have behavioral characteristics that contribute to the infection by *Leishmania infantum*, and as such, FeL has been reported worldwide. When clinical signs of FeL are present, they are usually cutaneous, with unspecific dermatological changes, that frequently occur in other feline diseases and if not diagnosed can contribute to an underestimation of the actual occurrence of the disease in cats. The low seroprevalence titers along with the commonly asymptomatic infection in cats can further contribute to the underestimation of FeL occurrence.

This work aims to bring up to date the current status of FeL infection worldwide. It comprises a review of the most recent case reports and surveillance studies. Although currently limited, the most relevant and recent information on the parasite, vector, epidemiology, pathology, and immune response is presented, as well as available diagnostic and treatment strategies. The knowledge of the epidemiological and immunopathological features of FeL, in some aspects so different from the Canine Leishmaniasis (CanL), can be used as a tool in an attempt to prevent infection and decrease the hazard that FeL can embody for both humans and cats.

Key words:

Leishmania infantum; Cat; Epidemiology; Immunopathology; Diagnosis; Prevention

1. Introduction

The natural transmission of Leishmaniasis is classified as zoonotic, involving vertebrate hosts who function as reservoirs, being humans' accidental hosts; or anthroponotic, in which the epidemiologic cycle is established between the vectors and the human beings, with the later acting as reservoirs (Quinnell and Courtenay, 2009). Several *Leishmania infantum* hosts have already been identified, namely bats (Savani *et al.*, 2010), iberian hares (*Lepus granatensis*), wolfs (*Canis lupus*), red foxes (*Vulpes vulpes*) (Criado-Fornelio, 2000), egyptian mongooses (*Herpestes ichneumon*), genets (*Geneta geneta*), iberian lynx (*Lynx pardinus*), pine martens (*Martes martes*) (Ruiz-Fons *et al.*, 2013), crab-eating foxes (*Cerdocyon thous*) (Catenaci *et al.*, 2010), horses (Koehler *et al.*, 2002; Rolão *et al.*, 2005; Gramiccia, 2011), rats (Akhavan *et al.*, 2010), and lions (*Panthera leo*) (Dahroug *et al.*, 2011). Pigs that apparently resist to the infection, produce protective antibodies against the different antigens of *Leishmania infantum*, and thus are able to keep the parasitized phlebotomines in the surrounding geographic area (Moraes-Silva *et al.*, 2006).

Leishmaniasis in domestic cats (*Felis catus domesticus*) was described for the first time in 1912, in Algeria, in a cat that lived with a dog and a child, both infected with Leishmaniasis (Longoni *et al.*, 2012). Since then, Feline Leishmaniasis (FeL) has been reported globally, but more frequently in countries bordering the Mediterranean sea (Solano-Gallego, *et al.*, 2007), namely Spain (Navarro *et al.*, 2010; Millán *et al.*, 2011), France (Bourdoiseau, 2011; Pocholle *et al.*, 2012), Italy (Poli *et al.*, 2002; Spada, *et al.*, 2013) and Greece (Diakou *et al.*, 2009). FeL was also reported in other neighboring countries like Portugal (Maia *et al.*, 2008, 2010; Marcos *et al.*, 2009; Cardoso, *et al.*, 2010; Vilhena *et al.*, 2013), Israel (Nasereddin *et al.*, 2008), Switzerland (Rüfenacht *et al.*, 2005), and Iran (Hatam *et al.*, 2010). In the American continent, FeL was reported particularly in Central America (Trainor *et al.*, 2010), Brazil (Vides *et al.*, 2011; Sobrinho *et al.*, 2012), and Paraguay (Velázquez *et al.*, 2011).

According to the World Health Organization (WHO), Leishmaniasis' endemic areas have spread and the prevalence of the disease has increased, as well as the number of reported cases (Ready, 2010). Europe is one of the most affected continents concerning the risk of re-emergency of the disease by several reasons: appearance of exotic *Leishmania* species (motivated by the increased mobility between some countries); increased population density,

associated with factors that cause immune-depressed populations (Brachelente *et al.*, 2005); resistance of parasites and vectors to the drugs and insecticides used; and, finally, dissemination of Leishmaniasis to neighboring countries, that present tempered climates, as a result of environmental and social-economic changes, as well as human and animal migration movement (Solano-Gallego *et al.*, 2007; Campino and Maia, 2010).

In Portugal, current regulation (*Decreto-lei* nº 314/2003, December 17th) establishes the National Program for the Control and Epidemiological Surveillance of Animal Rabies and other Zoonosis (PNLVERAZ), defining Leishmaniasis as a “zoonosis of risk”. Indeed, and similarly to other countries of Southern Europe, Portugal has registered increasing cases of Human Leishmaniasis (HuL). Between 2000 and 2009, the Unit of Leishmaniasis of the Institute of Hygiene and Tropical Medicine, in Lisbon, identified 173 new human cases of Visceral Leishmaniasis (VL). Among these 173 cases, 107 were related to immunosuppressed people, with concomitant Human Immunodeficiency Virus (HIV) infection. The remaining were adult and children. Previously, Leishmaniasis was considered predominantly a child disease (Campino and Maia, 2010). Concerning the Public Health impact of the Canine Leishmaniasis (CaL), FeL must also be considered in the veterinary practice, and its control should be implemented, appealing to pharmacological strategies (Maroli *et al.*, 2007).

The clinical diagnosis of FeL is hampered by the non-pathognomonic clinical signs in cats and the fact that some are asymptomatic. Furthermore, it is noteworthy that cats have been considered as uncommon hosts for *Leishmania* (Poli *et al.*, 2002; Martín-Sánchez *et al.*, 2007; Bresciani *et al.*, 2010; Longoni *et al.*, 2012).

This work aims to bring up to date the current status of FeL infection worldwide. It comprises a review of the most recent case reports and surveillance studies. Although currently limited, the most relevant and recent information on the parasite, vector, epidemiology, pathology, and immune response is presented, as well as available diagnostic and treatment strategies. The knowledge of the epidemiological and immunopathological features of FeL, in some aspects so different from the CanL, can be used as a tool in an attempt to prevent infection and decrease the hazard that FeL can embody for both humans and cats.

2. The Parasite

Leishmaniasis is a parasitic disease caused by an obligate intracellular protozoan, of the genus *Leishmania* (Kinetoplastida, Trypanosomatidae) (Solano-Gallego and Baneth, 2006).

Species from genus *Leishmania* identified as infective for felines are subdivided in two subgenus: *Leishmania* (that includes the species characteristic from Old World, namely *L. major*, *L. infantum*, *L. donovani*, and that found in the New World: *L. mexicana*, *L. amazonensis*, *L. venezuelensis*) and *Viannia* (only occurring in Central and South America, e.g. specie *L. (Viannia) braziliensis*) (Killing-Kendrick, 2002; Martín-Sánchez *et al.*, 2006; Solano-Gallego and Baneth, 2006; Trainor *et al.*, 2010).

Leishmania infantum is also known as *L. chagasi* (Tomás and Romão *et al.*, 2008; da Silva *et al.*, 2008) despite the suggestion that *L. infantum* and *L. chagasi* are distinct species (Gramiccia and Longoni, 2005). Some lineages of *Leishmania infantum* are identified by DNA sequencing, monoclonal antibody reactivity to membrane antigens and also by the typification of isoenzymes throw electrophoresis. The last one allows the classification of lineages of *L. infantum* in zymodemes (MON), which result from different migration patterns of the isoenzymes according to the enzymatic systems present (Solano-Gallego and Baneth, 2006; Pennisi and Solano-Gallego *et al.*, 2013). Several zymodemes have already been described in *Leishmania infantum*, being MON-1 the most frequently reported and assumed as the responsible for zoonotic Leishmaniasis, affecting humans, canines, felines and other hosts. In fact, the majority of the clinical cases of FeL caused by *Leishmania infantum* reported in Europe belongs to zymodeme MON-1 (Ozon *et al.*, 1998; Pratlong *et al.*, 2004; Grevot *et al.*, 2005; Cardoso *et al.*, 2010; Maia *et al.*, 2010; Gramiccia, 2011).

Besides MON-1, Pratlong *et al.* (2004) identified further six zymodemes (MON-11, MON-24, MON-29, MON-33, MON-34, and MON-108) which differ from MON-1 in only one of three enzymatic systems. Zymodemes seem to be related with the genetic expression of Leishmaniasis. The existence of polymorphism in some enzymatic systems of *Leishmania infantum* is suggested as relevant in the clinical expression of the illness. Such polymorphisms can result from genetic exchanges between the parasites or, according to other hypothesis, from translational or posttranscriptional modifications of certain enzymes codified by a multigenic family.

During its lifecycle, *Leishmania* presents two main forms: promastigote (fusiform and flagellated living in the phlebotomine vector) and amastigote (ovoid with intracellular flagella infecting the host macrophages). Protozoan from genus *Leishmania* replicates by binary division, initiated in the kinetoplast, followed by flagella and nuclei, respectively. Some authors describe the rare existence of sexual reproduction phenomena (Tomás and Romão, 2008).

Female sand flies acquire *Leishmania* parasites when they feed on an infected vertebrate host in search of a blood meal. During feeding, sand fly mouth parts cause injury in the host's skin and *Leishmania* amastigotes are transferred from the infected vertebrate being ingested by the vector. In the gut of the vector, *Leishmania* amastigotes develop into *Leishmania* promastigotes which multiply quickly by binary division.

A process apparently mediated by lipophosphoglycans fix the promastigotes to the internal surface of the abdominal midgut (Killick-Kendrick, 2002).

The structure of the membrane glycoconjugate of the promastigotes varies according to the *Leishmania* species, considering that union of the parasite to intestinal cellular membrane requires molecular specificity, depending on the arthropod species. According to Killick-Kendrick (2002) lecithin, which exist in increased values in the female intestines, could act for molecular adhesion factor.

Initially, the parasite is confined inside of a peritrophic membrane produced by the arthropod intestinal cells, differentiating in procyclic promastigotes. Production of chitinolytic enzymes causes their release into the intestinal lumen. These procyclic promastigotes develop successively into metacyclic promastigotes losing the ability to replicate. An intermediate form, called haptomonad promastigotes fix to stomodaeal valve through hemidesmosomes, allowing that metacyclic promastigotes reach the oral cavity. Salivary glands are not colonized, and promastigotes are inoculated through regurgitation, when the female takes a meal. *Leishmania* amastigotes occur inside the vertebrate host's macrophages and dendritic cells. These forms can infect neutrophils in a first instance, but their replication occurs inside macrophages (Tomás and Romão, 2008).

3. Vectors Bio-ecology

Phlebotomine sand flies measure on average 3 mm long and move in an area with about 1 km from the breeding site. Their activity occurs mainly at night and twilight avoiding windy conditions. In Mediterranean region the activity occurs between May and October when the temperature ranges from 15 to 28 °C. In tropical climate countries, the sand flies have activity during the entire year (Solano-Gallego and Baneth, 2006; Solano-Gallego and Cardoso, 2013).

The vectors of *Leishmania* spp. belong to the genus *Phlebotomus* (Diptera, Psychodidae) in Old World, and *Lutzomya*, in the New World (Afonso and Alves-Pires, 2008; Simões-Mattos *et al.*, 2004). The main vectors of this parasitosis in Portugal are *Phlebotomus perniciosus* Newstead, 1911, and *P. ariasi* Tonnoir, 1921 (Maia *et al.*, 2009). However, transmission of *Leishmania* in the Old World is probably not exclusive by arthropods from the genus *Phlebotomus*, given that recently, in Portugal, Campino *et al.* (2013) reported the occurrence of *Leishmania major* in sand flies from *Sergentomya minuta* specie, in Algarve region. This finding suggests that *L. major* circulating in Portugal represents a risk of introduction of new species in Portugal from North Africa and India given that Human CL (Cutaneous Leishmaniasis) caused by *L. major* occurs in the Eastern Mediterranean region, from Morocco to Afghanistan.

Human CL cases have been described in Portugal since 1940s. The cases are mostly located in the watershed of the rivers Douro, Tejo and Sado (Campino and Maia, 2010), and thus this zoonosis occurs in moist areas with 45-80 % relative humidity, despite the life cycle of the vector does not include the water (Solano-Gallego and Cardoso, 2013).

Some phlebotomines species show feeding preferences. Maroli *et al.* (2007) considered that the feeding preference of the female of *Phlebotomus sergenti* was cats and birds, while the feeding preference of the female of *Phlebotomus papatasi* was cats and dogs.

The occurrence of *L. infantum* autochthonous cases in the North Europe, in regions of high latitude, where the occurrence of phlebotomines was not described, support the theory that other vectors might be involved in the transmission (Quinnell and Courtenay, 2009). Indeed, in laboratory conditions it was demonstrated that *Rhipicephalus sanguineus*, the brown dog tick, was able to transmit *L. infantum* to rodents. Moreover, a transtadial transmission of *L. infantum* was demonstrated in *R. sanguineus*, more specifically in a nymph that became

infected after feeding in an infected dog and that remained infected in the adult stage after feeding in a non-infected dog (Morais *et al.* 2013).

Similar reports were described in fleas and lice. Authors detected *L. infantum* in 58.62 % of *Ctenocephalides felis* (Morais *et al.*, 2013). Nevertheless, the role of these ectoparasites in the transmission should not be neglected, and eventually they should be proposed as sentinels, on the spreading of *L. infantum* in risk areas, constituting, thus, a surveillance method of this parasite.

However, the role of these ectoparasites as vectors is controversial, with some authors arguing that the presence of the protozoan genetic material does not imply ability to transmit *Leishmania* to the vertebrate hosts (Killick-Kendrick, 2002; Solano-Gallego and Baneth, 2006).

4. Epidemiology of Feline Leishmaniasis

In European countries, *Leishmania infantum* is the responsible agent for the zoonosis (Tomás *et al.*, 2008; da Silva *et al.*, 2008). However, anthroponotic CL caused by *Leishmania tropica*, occurs sporadically in Greece. More recently, another species, *L. donovani*, considered anthroponotic, has been reported in Cyprus, causing Leishmaniasis in both cutaneous and visceral forms (Gradoni, 2013). Previously, in an epidemiological study carried in the Nepal, Deoxyribonucleic Acid (DNA) from *Leishmania donovani* was found in the blood of goat, bovine and buffalos (Bhattarai *et al.*, 2010).

Recent cases and studies involving the occurrence of *Leishmania* in cats suggest that these animals act as reservoirs (Solano-Gallego *et al.*, 2007; da Silva *et al.*, 2008; Gramiccia, 2011). The discussion regarding the classification of cats as accidental or alternative hosts, primary or secondary reservoirs continues (Solano-Gallego *et al.*, 2007; da Silva *et al.*, 2008; Navarro *et al.*, 2010; Gramiccia, 2011).

According with the WHO classification, three types of hosts exist: primary, secondary and accidental. The vertebrate mammals of the orders Primate (e.g. humans) and Carnivore (e.g. dogs, foxes, wolves) are considered primary hosts (Longoni *et al.*, 2012).

The classification of a host as primary, secondary (Sín. minor) or accidental is based in the capacity of *Leishmania* species to persist, indefinitely or temporarily in a population that is reservoir of the disease, characterized by a basic reproduction number (R_0). Quinnell and

Courtenay (2009) claim that the R_0 value must be higher than 1 to confirm the animal as a main host. The main host is responsible for transmitting indefinitely the agent in the nature and is often asymptomatic. Two main hosts are able to exist and operate in the same area, linking the domestic and sylvatic cycles.

According to the same authors, to verify the condition of secondary hosts, the R_0 is increased. Although a secondary host can transmit the infection, it does not guarantee the transmission without the presence of the primary host(s). Accidental hosts can be also infected, but usually they do not transmit the parasite, and thus do not influence the R_0 value.

Available data from epidemiological surveys (Table 1) and case reports (Table 2) suggest that the cat can act as an alternative host of *L. infantum*, and not as an accidental host, for several reasons, as follows (Gramiccia and Gradoni, 2005; Grevot *et al.*, 2005; Maroli *et al.*, 2007; Maia *et al.*, 2010): 1) cats can be infected and do not develop illness; even if they present clinical signs, a chronic presentation will ensue; 2) in the peripheral blood of cats, the protozoan is in the infective form for the vector, i.e. they are infecting for the vector; 3) cats cohabit with human beings, namely in endemic areas of CaL; 4) sick cats infected with *Leishmania* do not recover without anti-*Leishmanial* therapy.

Cats have behavioral characteristics that can contribute to exposure: they are nocturnal predators, operating in a 1.5 km radius from its residence, being able to use forests as a hunting territory and, therefore, being ideal elements to link the sylvatic and domestic cycles, favoring the dissemination of the parasite (da Silva *et al.*, 2008). Thus some authors claim that cats can be considered as disease amplifiers (Maia *et al.*, 2009), which renders further importance to the assessment of the epidemiological role of the cat through further studies.

Table 1. Compilation of worldwide epidemiologic surveys of Feline Leishmaniasis due to *Leishmania infantum*.

Country (region)	Seroprevalence (total number of samples)	Diagnostic Assay	Confirmatory Assay: Results	Reference
ITALY (Milan)	25.3% (233)	IFI	qPCR: 0%	Spada <i>et al.</i> (2013)
BRAZIL (Araçatuba)	4.64% (302)	IFI	ELISA: 12.91% Direct Parasitological Exam: 9.93%	Sobrinho <i>et al.</i> (2012)
MEXICO (Yucatan Peninsula)	22.1% (95)	ELISA (Fe-SOD)	-	Longoni <i>et al.</i> (2012)
PARAGUAY (Asuncion)	0.94% (317)	IFI	-	Velásquez <i>et al.</i> (2012)
BRAZIL (Araçatuba)	25.4% (55) 10.9% (55)	ELISA IFI	-	Vides <i>et al.</i> (2011)
IRAN	25%(40)	IFI DAT	-	Hatam <i>et al.</i> (2010)
PORTUGAL (Lisbon)	1.3%(142)	IFI	PCR: 20.3 % (28/138)	Maia <i>et al.</i> (2010)
PORTUGAL (North region)	2.8% (316)	DAT ELISA	-	Cardoso <i>et al.</i> (2010)
GREECE (North region)	3.87% (284)	ELISA	-	Diakou <i>et al.</i> (2009)
ISRAEL (Jerusalem)	6.7%(104)	ELISA	-	Nasereddin <i>et al.</i> (2008)
PORTUGAL (Lisbon)	20% (20)	IFI	PCR: 30.4 % (7/23)	Maia <i>et al.</i> (2008)
SPAIN (South region)	60% (183) with title ≥ 10 28.3% (183) with title ≥ 40	IFI	PCR- ELISA: 25.7 % Direct Parasitological Exam: 3 of 7 tested positive	Martín-Sánchez <i>et al.</i> (2007)
ITALY	16.3% (203)	IFI	-	Vita <i>et al.</i> (2005)
ITALY	0.9% (110)	IFI	-	Poli <i>et al.</i> (2002)
FRANCE	12.4% (97)	Wb	-	Ozon <i>et al.</i> (1999)

(DAT, Direct Agglutination Test; ELISA, Enzyme-Linked Immunosorbent Assay; Fe-SOD, Iron SuperOxide Dismutase;
IFI, Indirect Immunofluorescence; PCR, conventional Polymerase Chain Reaction; qPCR, Real-time Polymerase Chain Reaction; Wb, Western blot)

Table 2. Compilation of worldwide case reports of Feline Leishmaniasis due to *Leishmania infantum*.

Country (region)	Cat Identification	Diagnostic Assay	Reference
FRANCE (South, St. André Roche)	14-years-old male cat	Histopathology Western blot qPCR Blood culture	Pocholle <i>et al.</i> (2012)
PORTUGAL (Porto)	4-years-old female cat	Bone marrow cytology Buffy coat cytology PCR Indirect hemagglutination test	Marcos <i>et al.</i> (2009)
FRANCE (South, Biot)	6-year-old female cat	Histopathology Bone marrow cytology PCR	Ozon <i>et al.</i> (2005)
FRANCE (South, Grasse)	13-years-old neutered male cat	IFI ELISA Histopathology Western blot Blood culture	Grevot <i>et al.</i> (2005)
SPAIN (Barcelona)	8-years-old female cat	ELISA Ocular histopathology Bone marrow cytology PCR	Leiva <i>et al.</i> (2005)
BRAZIL (São Paulo)	2-years-old male cat	IFI PCR	Savani <i>et al.</i> (2004)
ITALY	14-years-old female cat	IFI, lesions cytology	Pennisi <i>et al.</i> (2002)
	6-years-old male cat	Lymph node cytology, PCR, IFI	
	10-years-old female cat	Lymph node cytology, PCR, IFI	
	Adult male cat	Lymph node cytology, PCR, IFI	
ITALY (Imperia)	6-years-old female cat	IFI Histopathology Lesion and lymph node cytology PCR Electron microscopy	Poli <i>et al.</i> (2002)
SPAIN	3-years-old female cat	IFI Popliteal lymph node cytology	Hervás <i>et al.</i> (1999)
	5-years-old female cat	Histopathology Electron microscopy	

(PCR, Polymerase Chain Reaction; ELISA, Enzyme-Linked Immunosorbent Assay; IFI, Indirect Immunofluorescence; qPCR, Real-time Polymerase Chain Reaction)

In a study carried in the North of Portugal (Alto-Douro-e-Trás-os-Montes region), considered endemic for CaL, Cardoso *et al.* (2010) analyzed serum of 242 felines, correlating factors as age, race, origin, gender, origin (agricultural or urban) and *habitat* (*indoor* or *outdoor*). Although they reported a low seroprevalence (2.8 %) when compared with similar studies, it was possible to observe that positive animals were adult or aged animals (between 24 and 204 months), males revealed significantly higher frequency of positivity (4.7 %) compared to the females (0.7 %). In addition, felines from rural environment were also more frequently infected (10.5 %). According to authors of this study, these findings probably result from a cumulative exposition to the infection, due to behavioral attitudes that cats present in their territorial and sexual exploring attitudes. The remaining studied factors (cats *habitat* regime - *indoor* or *outdoor*, clinical manifestation and breed) did not show any positive statistical correlation with the infection. Nasereddin *et al.* (2008) studied the relation between FeL infection and altitude, detecting 86 % (6/7) of seropositivity in the tested cats, resident above of the 762 meters.

In a study carried out in Portugal, using PCR (Polymerase Chain Reaction) assay, (Maia *et al.*, 2010), 18.5 % (17/92) of the cats and 31.4 % (43/137) of the dogs were positive during the no-transmission period for the sand fly, i.e. between October and May. During the transmission season, the number of positivity increased in both populations of cats (22.0 %; 11/50) and dogs (60.0 %; 9/15). However, and in accordance with the authors of the study, additional studies would be necessary to determine if the infection in these animals maintains, through reliable evaluation techniques, and considering more than one transmission season.

It is noteworthy that recent studies on CaL show the possibility of occurrence of other horizontal transmission form besides sand flies, namely through blood transfusion, as well as other vertical transmission form, through the placenta with occurrence of placentitis in VL (Gibson-Corley *et al.*, 2008; Boggiatto *et al.*, 2011). The infection in dogs can still be acquired through transplant of contaminated organs and needles (Morais *et al.*, 2013). Venereal transmission in dogs, until then considered impossible, was scientifically proven in a study of da Silva *et al.* (2008), by the presence of *Leishmania chagasi* in infected dog's semen. *Leishmania* amastigotes infect the female, due to mechanical trauma of the genitalia of the female, inherent to proper copula.

5. Pathogenesis and Lesions

According to the epidemiological surveys and case reports consulted, felines are usually asymptomatic (Solano-Gallego *et al.*, 2007; Nasereddin *et al.*, 2008; Maya *et al.*, 2010), what raises questions about their classification as accidental or alternative hosts, as discussed above. As a revealing example, in the study of Costa *et al.* (2010) entailing a population of 200 cats, only 2 animals revealed clinical signs, in the form of crusty lesions of the dorsal cervical region along with hepatosplenomegaly.

Overall, clinical signs of the disease are comprised in three main clinical forms. VL, caused by *L. donovani* and *L. infantum* is considered less common in cats. This clinical form is associated with high mortality and features a systemic involvement of the organism. CL and Mucocutaneous Leishmaniasis (MCL) caused by *L. major*, dermatropic *L. infantum*, and *L. brasiliensis* are associated with significant morbidity, compromising the tegumentar and mucosal systems (Hervás *et al.*, 1999; Navarro *et al.*, 2010; Gramiccia, 2011).

The first reported cases of FeL were characterized by cutaneous manifestation, without visceral involvement (Bonfante-Garrido *et al.*, 1996; Ozon *et al.*, 1998; Hervás *et al.*, 1999; Pennisi, 2002; Bourdoiseau, 2011), with dry local lesions, as papules and nodules, and exudative lesions, as crusts and ulcers (Maroli *et al.*, 2007; Gramiccia, 2011). The importance of the screening of cats presenting dermatitis, nodular or ulcerative was further demonstrated by Navarro *et al.* (2010). In this study, 15 cats infected with leishmaniasis presented the cutaneous expression of the illness, namely skin lesions in mucocutaneous junction (nose, lips and ears) as well as ocular lesions. Coelho *et al.* (2011) also described granulomatous perifolliculitis, dermatitis lichenoid and pododermatitis in cats with Leishmaniasis. VL is considered little common in the cats. Similarly, in the South of France, Pocholle *et al.* (2012), described a clinical case of a 14-years-old Feline Immunodeficiency Virus (FIV) positive cat, with a 3-year history of recurrent pododermatitis, not responsive the antibiotics and characterized by exudative and erythematous lesions. Besides a 20 % weight loss, the cat presented three cutaneous injuries (at the base of the ear, head, and interscapular region), all with aspect of ulcerated or hemorrhagic papules, but circumscribed. A fourth lesion in the auricular pavilion was identified, compatible with carcinoma of the squamous cells.

Clinical manifestations entailing lymphadenomegaly were also reported. Maroli *et al.* (2007) described a cat with FeL, presenting as the only clinical sign lymphadenomegaly of the mandibular lymph node and mild periodontitis. In the case reported by Denuzière (1976), beyond lymphadenomegaly, the cat presented fever. In Spain an infected female cat presented lymphadenomegaly in addition to dermatological lesions as scaling and alopecia of head and abdomen, as well as ulcers in the bone prominences. History of abortion was also referred (Hervás *et al.*, 1999). Located lymphadenopathy of the popliteal lymph node, along with onicogriphosis, cachexia with muscular atrophy, and weakness were described in a cat with a left pinna wound (da Silva *et al.*, 2010).

Ocular Leishmaniasis also was reported, with ocular lesions such as corneal exudative ulcers, panuveitis and panoftalmatitis (Leiva *et al.*, 2005; Navarro *et al.*, 2010).

Although more seldom, cats with VL, but without cutaneous signals have also been referred, presenting at clinical examination fever, jaundice, vomits, lymphadenomegaly, lesions of the oral mucosa with gingivitis, anemia, leukopenia (Leiva *et al.*, 2005; Maroli *et al.*, 2007; Marcos *et al.*, 2009).

Renal failure associated with FeL seems less evident than in dog, among which it is a well-recognized syndrome and a cause of death. Navarro *et al.* (2010) identified a feline that died with renal insufficiency, and two that developed the same clinical picture, few months after being diagnosed with Leishmaniasis.

Some investigators further propose a synergism between squamous cells carcinoma and FeL, claiming that while the carcinoma could take advantage of the proliferation of the protozoan, the parasite could initiate the development of the neoplasia. The primary cause of dermal ulcers can be the protozoan, the neoplasia, or even both. Lesions compatible with squamous cells carcinoma were described in the left temporal region of a 13-years-old cat (Grevot *et al.*, 2005) and in the auricular pavilion of a 14-years-old cat (Pocholle *et al.*, 2012), both FIV-positive.

It is noticeable that FIV and/or Feline Leukemia Virus (FeLV) infections were referred, during some time, as predisposing factors to the infection by *Leishmania infantum* explained by the ensuing immunosuppression (Pennisi *et al.*, 2004; Simões-Mattos *et al.*, 2005; Costa *et al.*, 2010).

Supporting studies include the one of Pennisi *et al.* (2002) that found 70 % of cats positive to Leishmaniasis and FIV. Similarly, Sobrinho *et al.* (2012) found a positive association between FeL and FIV infection in 70.59 % of the tested cats.

In a study lead by Sherry *et al.* (2011), in the Ibiza Island, infection by *Leishmania* and FeLV also featured a statistical correlation.

However, the results of some studies contradict this positive correlation between FIV and/or FeLV and FeL infection (Ozon *et al.*, 1998; Savani *et al.*, 2004; Vita *et al.*, 2005; Maroli *et al.*, 2007; Solano-Gallego *et al.*, 2007; Marcos *et al.*, 2009; Maia *et al.*, 2010; da Silva *et al.*, 2010; Bourdoiseau, 2011; Coelho *et al.*, 2011). In Brazil, Savani *et al.* (2004) reported the first FeL autochthonous case, in a 2-years-old cat that was FIV- and FeLV-negative, but positive to Feline Infectious Peritonitis (FIP). The cat presented a nodular lesion in its nose and visceral commitment.

Regarding *Toxoplasma gondii*, of which cats are considered reservoirs, the majority of the studies did not observed a positive correlation between both infections (Nasereddin *et al.*, 2008; Cardoso *et al.*, 2010; Coelho *et al.*, 2011; Sherry *et al.*, 2011).

Finally, the relation of FeL with infections by *Trypanosoma cruzi* was studied by Longoni *et al.*, (2012) in a population of 95 stray cats of the Yucatan Peninsula, in Mexico. The seropositivity was generally considered low, but they found co-infection of *T. cruzi* with three *Leishmania* species, specifically *L. mexicana* (10.5 %), *L. brasiliensis* (11.75 %), and *L. infantum* (22.1 %).

6. Immunological Features of *Leishmania*

It is well ascertained that in the canine model, the parasite depresses the innate immune defense of the host and survives in the interior of phagolysosomes, in the interior of the infected macrophages, producing lipophosphoglycans (Solano-Gallego and Baneth, 2006). Thus, in CaL an effective immune response requires Cell-Mediated Immunity (CMI), being macrophages the responsible ones in the control of the infection by *Leishmania*.

As depicted in Figure 1, the activated Type-1 T-helper cell (Th1) induce the anti-*Leishmania* activity of macrophages through secretion of cytokines such as interferon gamma (IFN- γ), interleukin-2 (IL-2) and alpha tumoral necrosis factor (TNF- α). The nitric oxide (NO) produced by the macrophages is the main mediator molecule of the intracellular destruction of the

amastigotes forms, through cellular apoptosis, controlled by inhibition of proteasomes. In contrast, interleukin-10 (IL-10), interleukin-4 (IL-4) and the transformation growth factor beta (TGF- β) are involved in the dissemination of the protozoan, leading to the increase of B-cells and T-cells, with associated hyperglobulinemia. The IL-10 is produced by regulating cells T (Treg) which inhibit Th1 cells. Nonetheless the IL-10 is also produced by the Th1 cells itself, with a limiting beneficial effect in the pathology by keeping low levels of infection (Baneth *et al.*, 2008).

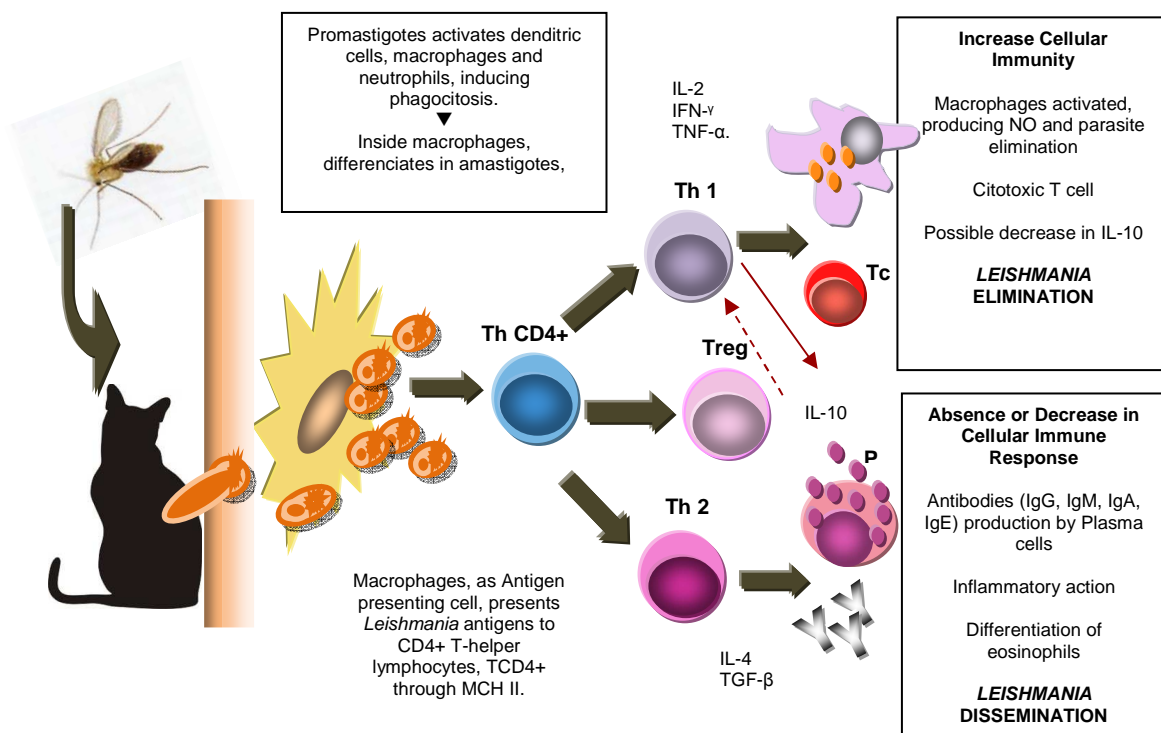


Figure1. Suggested immune response of felines in *Leishmania* infection, according with immune mechanism in CaL (modified from Barbiéri, 2006; Baneth *et al.*, 2008; Saz *et al.*, 2013) (IFN- γ , Gamma Interferon; Ig, Immunoglobulin; IL, Interleukin; MCH II, Major Histocompatibility Complex; NO, Nitric Oxide; P, Plasma Cell; Tc, Cytotoxic Cell; TCD4+, T-helper Cell; Th1, Type-1 T-helper cell; Th2, Type-2 T-helper Cell; ; TGF- β , Transformation Growth Factor Beta; TNF α , Alpha Tumor Necrosis Factor; Treg, Regulatory T-cell).

The immune response in the VL in human beings, canines and rodents, does not feature a standard Th1/Th2 dichotomy. This balance is determinant in the response of the organism; control of the parasite and resistance to the disease occurs if immune response is mediated

predominantly by Th1 cells; clinical manifestation and clinical regression is common among immune responses mediated predominantly by Th2 cells).

According to *in vivo* and *in vitro* studies, CD4+ Th cells are the ones that confer the protective immunity against Leishmaniasis. However, some studies indicate CD8+ Th cells as responsible for asymptomatic infection of dogs and resistance to *Leishmania* infection (Barbiéri, 2006).

Leishmaniasis in cats seems to involve the cellular immune response, with activation of macrophages for the destruction of the intracellular forms amastigotes. The high antibodies titers, usually present in some dogs and also in some symptomatic cats, do not confer immunity against the disease (Barbiéri, 2006). Nevertheless, some investigations showed that animals with increased titers of anti-*Leishmania* antibodies presented decreased positivity for the presence of DNA in the PCR methodology whereas the biggest positivity of PCR occurred more frequently in cats with reduced antibody titers (Martín-Sánchez *et al.*, 2007; Costa *et al.*, 2010). This corroborates the hypothesis that immune response in felids differs from the one observed in dogs, justifying the high number of asymptomatic infected cats, and the variable clinical manifestation of the disease, showing that lesions occur before the production of antibodies. When these lesions are in a resolution phase, seroconversion occurs, what could suggest that humoral immune response is protective in FeL. On the other hand, it shows that conventional serological methods to detect active infection in cats are not always reliable (Martín-Sánchez *et al.*, 2007).

The natural resistance of cats to Leishmaniasis is widely suggested by the spontaneous healing of the lesion, which is often characterized by minimal or limited pathological changes (Simões-Mattos *et al.*, 2005; Navarro *et al.*, 2010). The parasite dissemination and its interaction with the host's immune system is reflected in organic changes such as lymph node hypertrophy due to the proliferation of blastic lymphoid cells in lymphoid follicles. If the immune response is not cellular, the proliferative reaction will involve macrophages, plasma cells, reticular cells migration, with decrease in the number of lymphocytes. Hyperplasia of the connective tissue occurs when the process becomes chronic, noticeable with fibrosis. Granulomas are rich in macrophages, granulocytes and T-cells, representing a good immune response. Dermatological ulcer can ensue, with cellular cytotoxic phenomena, or alternatively with deposition of immune complexes and organ hyperplasia (Santos *et al.*, 2008).

7. Diagnosis

Laboratory methods are important for the diagnosis of *Leishmania* infection, as a complement of the physical examination. Particularly in clinically manifested VL, hemogram and biochemical analysis frequently show leukocytose with neutrophilia, as well as urea and aspartate aminotransferase above of the values of reference. Creatinine, alanine aminotransferase and alkaline phosphatase can present normal values (da Silva *et al.*, 2010). Neutrophilia, with monocytosis and hyperglobulinemia with polyclonal gammopathy was also reported (Leiva *et al.*, 2005).

Laboratory methods of diagnosis of infection are generally classified as direct or indirect. A direct method will detect the parasite or some of its parts or antigens. Examples include PCR, Restriction Fragment Length Polymorphism (RFLP), immunohistochemistry, observation of the parasite in culture, in smears and in histologic sections of organs infected by *Leishmania*. An indirect method will detect the immune response against the parasite, whether the humoral response e.g. IFI, Indirect-Enzyme-Linked Immunosorbent Assay (ELISA), Direct Agglutination Test (DAT) or the cellular response (e.g. Delayed Hypersensitivity Test – DHT).

The direct observation of the parasite might be done through cytology and/or skin biopsy, namely from cutaneous lesions, lymph node or bone marrow (Ozon *et al.*, 1998; Costa *et al.*, 2010). Cytology, by aspiration or impression, can be carried out during the necropsy (Bresciani *et al.*, 2010), in affected organs with VL as liver, spleen and kidney (Grevot *et al.*, 2005; Marcos *et al.*, 2009). Recently, Costa *et al.* (2010) demonstrated that direct parasitological examination of popliteal lymph node by aspiratory cytology was more sensitive when compared with the cytology from other organs, such as the bone marrow, spleen or liver.

Marcos *et al.* (2009) found *Leishmania infantum* amastigotes in the cytoplasm of neutrophils in both blood and buffy coat smears (4 % of the neutrophils).

In Portugal, a case of VL was reported in a 4-years-old domestic short-hair neutered female cat confirmed as FIV- and FeLV-negative (Marcos *et al.*, 2009). After necropsy, *Leishmania infantum* amastigotes forms were found in the buffy coat and bone marrow smears, as well as in the splenic parenchyma and the follicular centers of lymph nodes.

Histopathology is referred as a method with an acceptable sensitivity and specificity for the diagnosis, especially in cats with cutaneous lesions. Pocholle *et al.* (2012) found *Leishmania*

amastigotes through histopathologic examination of cutaneous lesions in cats even when there was no suspicion of Leishmaniasis. Furthermore, immunohistochemistry techniques can be used for confirmation of histopathological examination (Navarro *et al.*, 2010), or as first line diagnosis (Vides *et al.*, 2011).

The culture of *Leishmania* promastigotes is an additional direct method, but it has some disadvantages as it features a low sensitivity and is time consuming, taking too long to get the results (Pocholle *et al.*, 2012). When employed, the samples used for culture are blood, bone marrow or lymph nodes. Nevertheless, some authors (Martín-Sánchez *et al.*, 2007) consider that blood is a not suitably sensitive specimen for culture in cats, because of the low parasitaemia and small amount collected for this purpose, resulting in lower sensitivity of the culture method.

The traditional methods such as cytology and culture are less sensitive than the new molecular methods and do not allow the identification of *Leishmania* species (Akhavan *et al.*, 2010). The established higher sensitivity of molecular techniques such as PCR, makes it a good option to confirm the diagnosis and for the detection of asymptomatic animals (Gramiccia and Gradoni, 2005). PCR is a DNA amplification technique of a specific fragment in a complex mixture by multiple cycles of DNA synthesis from oligonucleotide primers followed by short thermal treatments to separate the complementary strands. Nevertheless, the detection of DNA of *Leishmania* does not necessarily mean the existence of an active infection. Recently Morais *et al.* (2013) showed some advantages of Real time PCR (qPCR) as compared with conventional PCR (cPCR). In qPCR fluorescent report dyes are used to combine the steps of amplifications and DNA quantification in a single tube format, being the results given in real time. The advantages of qPCR include higher sensitivity and specificity, faster execution, and possibility of quantification of the DNA. Indeed, in the mentioned study of Morais *et al.* (2013) the samples with low parasitic load gave negative results in cPCR, but qPCR confirmed its positivity. The most suitable sample to detect the DNA of *Leishmania* is material obtained from puncture of lymph nodes (Maia *et al.* 2010).

RFLP is a molecular technique that is used in association with PCR, to compare the different electrophoretic patterns of restriction fragments (amplicons after digestion with restriction enzymes). With this molecular technique the variations in homologous DNA sequences are

analyzed and recognized, which allows protozoan classification in zymodemes (Dahroug *et al.*, 2011; Ruiz-Fons *et al.*, 2013).

Regarding the indirect methods for FeL diagnosis, the most important ones are the serological techniques, i.e. assays that detect antibodies. Examples of serological assays already used in epidemiological surveys (Table 1) and diagnosis of reported clinical cases (Table 2) of FeL include IFI, Western Blot (WB), indirect-ELISA and DAT. When considering serological assays, it is important to bear in mind that the cat's immune response against *Leishmania* differs from that observed in dogs, either by the number of infected animals, asymptomatic occurrence, or by the clinical signs of infected animals (Martín-Sánchez *et al.*, 2007; Costa *et al.*, 2010). In addition, and as depicted in Table 1 and further discussed in the previous section (*vide* section 6) feline serological surveys show a low seroprevalence of FeL, probably as consequence of the predominant cellular immune response in cats which results in low antibody titers or even in seronegativity (Marcos *et al.*, 2009; Maia *et al.*, 2010.). Nevertheless, some authors (Maroli *et al.*, 2007) refer that FeL has increased in the South of Europe and along with its corresponding seroprevalence rate, which although considered low in cats, has come closer to the rate reported in dogs in endemic areas. The use of cut-off antibody titers or even reagents produced specifically for dogs can further explain such low seroprevalence (Maroli *et al.*, 2007; Longoni *et al.*, 2012). As in all serological analysis, the time of sample collection is determinant. Maroli *et al.* (2007) described a cat infected with *Leishmania infantum*, which initially was seronegative, but presented seroconversion some time after the first serology.

One of the most important serological techniques is the IFI, also known as Indirect Fluorescence Antibody test (IFAT). IFI consists in the detection of antibodies anti-*Leishmania* which bind the target promastigote forms immobilized in a microscope slide. The presence of antibodies anti-*Leishmania* allows the visualization of the forms given that the secondary antibodies are bound to fluorochromes. The results are observable in a fluorescence microscope. The use of the promastigote forms as antigens renders specificity to the method, because these are the infectious form to the cat, inducing macrophage phagocytosis by specific union to their membrane molecules (Tomás and Romão, 2008). IFI is considered as highly sensitive in case of HuL (83.3 %) and CaL (87.5 %) (Maia *et al.*, 2008) However, IFI lacks sensitive in the diagnosis of *Leishmania* infection in cats, since the titers of antibodies anti-

Leishmania remain low. Thus although the IFI is considered the reference technique of CaL diagnosis, its use in FeL diagnosis is not consensual (Longoni *et al.*, 2012; Spada *et al.*, 2013). The suggested IFI cut-off titer was 1:80 for cats (Solano-Gallego *et al.*, 2013).

Another serological technique is the ELISA, which uses the principle of antigen-antibody interaction to detect antibodies (if used as a serological technique) or proteins, such as those making up cellular components of the parasite (if used as a direct method of diagnosis). In the study of Nasereddin *et al.* (2008) the antibody titers assessed through ELISA ranged from 1:2 to 1:200.

WB allows identification of specific target proteins. Proteins are first separated according to their molecular weight in an electrophoresis gel, and then identified by a specific directed antibody (Fonseca *et al.*, 2008). Longoni *et al.* (2012) in their ELISA and WB serological investigation techniques utilized a molecular specific marker, highly immunogenic, named superoxide dismutase of iron (Fe-SOD). This marker signals antibodies, allowing the identification of *Leishmania* species, without reported cross-reaction between them.

DAT is based in direct agglutination principle to detect specific anti-*Leishmania* antibodies. This technique uses *Leishmania* promastigotes, and react with serum antibodies. Serial two-fold dilutions are made (Cardoso *et al.*, 2010).

Additionally, positive DTH indirectly proves exposure to the parasite (Solano-Gallego *et al.*, 2000).

Interestingly, in an original strategy, Maroli *et al.* (2007) diagnosed FeL by the so called phlebotomy. Briefly, in a laboratorial and controlled environment, the patient was submitted to *Phlebotomus perniciosus* females bite, negatives for the *Leishmania*. After a 90 minutes meal, the sand flies were dissected and promastigote forms of the parasite were found, proving, for the first time, the natural infection to a competent vector of *L. infantum*, through a chronically sick feline patient. The xenodiagnoses of *Leishmania infantum* in a feline from Brazil was equally proven by da Silva *et al.* (2010) involving phlebotomy with *Lutzomyia longipalpis*.

8. Therapy and Prevention Strategies

The information about therapeutic efficacy in FeL cases is rare, with little investigated cases given that the majority of the anti-*Leishmania* drugs have been studied for dogs only. Even for

dogs, some of the studied and homologated treatment options are not considered as capable of complete cure (Solano-Gallego and Baneth, 2006). In addition, recently, Aït-Oudhia *et al.* (2012) claimed that the lineage of *Leishmania* is also related with the host's clinical manifestation, as it can modulate the susceptibility or determine resistance to one drug.

The naturally infected cats do not seem to recover without specific anti-*Leishmanial* therapy (Solano-Gallego *et al.*, 2007). However, in a study conducted by Martín-Sánchez *et al.* (2007), in Spain, a total of 27 cats, with FeL diagnosed by IFI and/or PCR, were monitored for 12 months, during which, eleven of them evidenced good clinical status without any treatment for *Leishmania*.

Drugs implemented in Leishmaniasis treatment are classified in two classes: leishmanistatics and leishmanicides. The first class includes allopurinol, a drug for gout / antimetabolite that inhibits the multiplication of parasites by inhibiting enzymes that are involved in the purine conversion, resulting in a decreased capacity of synthesis of Adenosine Triphosphate (ATP) and Ribonucleic Acid (RNA) (Corrales, 2013).

Leishmanicide includes drugs like meglumine antimoniate, or the new anti-*Leishmanial* drug miltefosine, also studied for breast cancer. Antifungals (clotrimazole, ketonazole, and amphotericin B), pentamidine, paromomycin (an aminoglycoside also known as amminosidine) and levamisole may also be used in this approach, producing a decrease in *Leishmania* load. The concomitant use of a leishmanicide and allopurinol accomplishes a synergic action (Solano-Gallego and Baneth, 2006; Corrales, 2013).

Denuzière (1976) administered 12 doses of pentamidine, IM, to a naturally infected cat, in the same dose recommended for the dogs. The cat reached clinical cure (Pennisi, 2002).

Recently, Navarro *et al.* (2010) mentioned a positive clinical evolution of the 2 cats treated with allopurinol (one with 7- and 14-years-old, with blepharitis and conjunctivitis, respectively, and both with raised parasitic load). Allopurinol, 100mg/daily, was also administered to a 14 years-old FIV-positive cat with history of recurrent pododermatitis for 3 years (case report described in the previous sections; Pocholle *et al.*, 2012). After 4 months of treatment, this case of disseminated Leishmaniasis was considered in regression, with healing of the dermic lesions. The treatment contributed, furthermore, to a reduction in the parasitaemia, presenting 11 parasites/mL in contrast with 26 parasites/mL at the beginning of the diagnosis. The cat died 3 months later in a

traffic accident and was submitted to necropsy. The *post-mortem* exam evidenced development of adiposity reservoirs, supporting the improvement of its clinical state. PCR confirmed the presence of parasites in the circulating blood.

In Spain, the successfully therapy administered to a cat with dermatological injuries and visceral commitment consisted in a combination of meglumine antimoniate 5mg/kg/SID, SC with ketoconazole, 10mg/kg/SID, PO. Treatment was followed by 3 cycles of 4 weeks, with 10 days interval (Hervás *et al.*, 1999; Solano-Gallego and Baneth, 2006). Already previously, the treatment with clotrimazole, followed of paromomycin 15%, topically implemented in a cat with *L. mexicana* infection, also with cutaneous lesions in the nose, was not effective. Six months later, the cat developed a new lesion in the nasal mucosa that was managed with levamisol (1mg/kg/48h), but without clinical success (Pennisi, 2002).

Some cats with CL have shown regression or positive clinical evolution, by revealing low number of parasites in lymphocytes and macrophages. Already cats with ocular, visceral and mucocutaneous junction lesions presented, in the cytological exam, a low number of lymphocytes along with an increased number of parasites in the interior of the macrophages, indicating a cellular reply and healing process (Navarro *et al.*, 2010).

A cat with visceral commitment, receiving support therapy and allopurinol (10mg/kg, PO, q12h), was euthanized on day 20 as reported by Marcos *et al.* (2009). Support treatment is required, especially in patients that develop visceral compromise, such as hepatic failure and chronic kidney disease, given the hepato- and nephrotoxic potential of some of the drugs. It is thus advised the monitoring of hepatic and renal functions in cats subjected to anti-*Leishmanial* therapy (Solano-Gallego and Pennisi, 2013).

Prevention is reiterated as the main goal, to make possible a timely diagnosis. Application of topical insecticides is a preventing mechanism of the sand flies bite. Repellents should also be used in animals that inhabit or travel, even if only temporarily, in endemic zones (Gradoni, 2013). Pyrethrins and pyrethroids are two recognized active principles that exert efficient repellent activity against phlebotomines. In the specific case of CaL control, this is a widely accepted and effective veterinary prophylactic measure. However, cats are sensible to pyrethrins and pyrethroids, because of the decreased hydrolysis of the esters of pyrethroids, which results in the existence of toxic metabolites that escape the hepatic degradation process.

Along with reduced glucuronidation, excretion of the same toxic metabolites from the feline organism is compromised (Stanneck et al., 2012a). Flumethrin, a recently available pyrethroid class molecule, features an excretion of its metabolites by fecal route, without commitment of the hepatic glucuronidation. It is thus assured the safe use of flumethrin in cats which is reported as effective against ticks, fleas and arthropods (Stanneck *et al.*, 2012b). Another tolerated active principle by cats is imidaclopride that, according to a study carried through in Italy, when combined with flumethrin showed efficacy in the prevention of CaL, in an area considered hyperendemic (Otranto *et al.*, 2013). Another prophylaxis measure recommended in the endemic areas of Leishmaniasis is the use of impregnated nets and the spraying of the shelters and spaces of the human beings and animals with insecticide solutions. The spraying with dichlorodiphenyltrichloroethane (DDT), long ago applied to prevent the plagues of arthropods in the agricultural regions and the dispersion of malaria and of Leishmaniasis, had been considered as efficient for this purpose, preferably when the vectors were on the shelters. However, given the threatening secondary effects of the DDT its use was forsaken.

The vaccination against the CaL is also possible nowadays, as an additional tool against Leishmaniasis (Quinnel and Courtenay, 2009). The development of immune cellular response seems to be dependent on the vaccine adjuvant, as well as the antigen combination utilized in the vaccine (Corrales, 2013).

Gradoni (2013) still evokes for the mandatory Leishmaniasis notification in the problematic regions, and also in the not endemic contiguous areas to the first ones.

9. Final remarks

The significance of the cat as a reservoir of *Leishmania* and not simply an alternative host seems to be gaining ground, mainly because: i) cats can present increased seropositivity between serology analysis; ii) cats can be infected during some months and thus are available for sand flies; iii) cats transmit the *Leishmania* agent in a competent form (Silva *et al.*, 2008). This possibility is supported by the recent report of autochthonous FeL cases (Petersen, 2009; Bourdoiseau, 2011).

The proximity of cats with human to the sand fly, and its susceptibility to the inoculated agent, allows infection, with a chronic evolution, and makes possible the survival of the animal until the

next feeding of the sand flies, thus contributing to the transmission of the agent (Maia *et al.*, 2008).

When clinical signs of FeL are present, they are usually cutaneous, with unspecific dermatological changes, that frequently occur in other feline diseases and if not diagnosed can contribute to an underestimation of the actual occurrence of the disease in cats (Poli *et al.*, 2002; Cardoso *et al.*, 2010). The low seroprevalence titers (Cardoso *et al.*, 2010) along with the commonly asymptomatic infection in cats can further contribute to the underestimation of FeL occurrence.

These reasons reinforce the need to raise awareness about FeL among veterinarians and to carry out further studies to broaden the knowledge on the significance of this disease in cats, and the actual epidemiological role of these animals in the transmission of this important zoonosis.

10. Conflict of Interest Statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

11. Acknowledgments

A special gratefulness to Doctor Carla Maia, for providing information and motivating the work in this field.

12. References

- Ait-Oudhia, K., Gazanion, E., Sereno, D., Oury, B., Dedet, J.P., Pratlong, F., Lachaud, L., 2012. In vitro susceptibility to antimonials and amphotericin B of *Leishmania infantum* strains isolated from dogs in a region lacking drug selection pressure. *Veterinary Parasitology* 187, 386-393.
- Afonso, M. O., Alves-Pires, C., 2008. Bioecologia dos vetores. In: *Leishmaniose Canina*. Merial, Lisboa, pp. 27-39.
- Akhavan, A.A., Mirhendi, H., Khamesipour, A., Alimohammadian, M.H., Rassi, Y., Bates, P., Kamhawi, S., Valenzuela, J.G., Arandian, M.H., Abdoli, H., Jalali-zand, N., Jafari, R.,

- Shareghi, N., Ghanei, M., Yaghoobi-Ershadi, M.R., 2010. *Leishmania* species: detection and identification by nested PCR assay from skin samples of rodent reservoirs. *Experimental Parasitology* 126, 552-556.
- Baneth, G., Koutinas, A.F., Solano-Gallego, L., Bourdeau, P., Ferrer, L., 2008. Canine leishmaniosis - new concepts and insights on an expanding zoonosis: part one. *Trends in Parasitology* 24, 324-330.
- Barbieri, C.L., 2006. Immunology of canine leishmaniasis. *Parasite immunology* 28, 329-337.
- Boggiatto, P.M., Gibson-Corley, K.N., Metz, K., Gallup, J.M., Hostetter, J.M., Mullin, K., Petersen, C.A., 2011. Transplacental transmission of *Leishmania infantum* as a means for continued disease incidence in North America. *PLoS neglected tropical diseases* 5, e1019.
- Bourdoiseau, G., 2011. Leishmaniose féline : actualités. *Pratique Médicale et Chirurgicale de l'Animal de Compagnie* 46, 23-26.
- Brachelente, C., Muller, N., Doherr, M.G., Sattler, U., Welle, M., 2005. Cutaneous leishmaniasis in naturally infected dogs is associated with a T helper-2-biased immune response. *Veterinary pathology* 42, 166-175.
- Bresciani, K., Serrano, A., de Matos, L., Savani, E., D'Auria, S., Perri, S., Bonello, F., Coelho, W., Aoki, C., Costa, A., 2010. Ocorrência d *Leishmania* spp. em felinos do município de Araçatuba. *Ver. Bras. Parasitol. Vet.*, v19, n2, 127-129.
- Bonfante-Garrido, R., Valdivia, O., Torrealba, J., Garcia, M., Garófalo, M., Urdaneta, I., Urdaneta, R., Alvarado, J., Copulillo, E., Momen, H., Grimaldi, G., 1996. Cutaneous leishmaniasis in cats (*Felis domesticus*) caused by *Leishmania venezuelensis*. *Revista Científica FCV-LUZ*, Vol VI, n 13, 187-190.
- Campino, L., Maia, C., 2010. Epidemiologia das leishmanioses em Portugal. *Acta Médica Port*, 23, 859-864.
- Campino, L., Cortes, S., Dionisio, L., Neto, L., Afonso, M.O., Maia, C., 2013. The first detection of *Leishmania major* in naturally infected *Sergentomyia minuta* in Portugal. *Memorias do Instituto Oswaldo Cruz* 108, 516-518.

- Cardoso, L., Lopes, A.P., Sherry, K., Schallig, H., Solano-Gallego, L., 2010. Low seroprevalence of *Leishmania infantum* infection in cats from northern Portugal based on DAT and ELISA. *Veterinary Parasitology* 174, 37-42.
- Cardoso, L., Solano-Gallego, M., 2013. Epidemiologia en Europa. In: Saz, S. V., Esteve, L. O., Corrales, G.M., Fondati, A., Giménez, M.T., Repiso, M.L., Freixa, C.N., Dantas-Torres, F., Otranto, D., Pennisi, M.G., *Leishmaniose: una revisión actualizada*, 1st Ed. Navarra: Servet editorial, pp 20-22.
- Catenacci, L.S., Giese, J., da Silva, R.C., Langoni, H., 2010. *Toxoplasma gondii* and *Leishmania* spp. infection in captive crab-eating foxes, *Cerdocyon thous* (Carnivora, Canidae) from Brazil. *Veterinary Parasitology* 169, 190-192.
- Coelho, W.M., do Amarante, A.F., Apolinario Jde, C., Coelho, N.M., de Lima, V.M., Perri, S.H., Bresciani, K.D., 2011. Seroepidemiology of *Toxoplasma gondii*, *Neospora caninum*, and *Leishmania* spp. infections and risk factors for cats from Brazil. *Parasitology research* 109, 1009-1013.
- Corrales, G., 2013. Tratamiento y pronóstico. In: Cardoso, L., Solano-Gallego, M., Saz, S. V., Esteve, G.M., Fondati, A., Giménez, M.T., Repiso, M.L., Freixa, C.N., Dantas-Torres, F., Otranto, D., Pennisi, M.G. *Leishmaniose: una revisión actualizada*, 1st Ed. Navarra: Servet editorial, pp 153-164.
- Costa, T., Rossi, C., Laurenti, M., Gomes, A., Vides, J., Sobrinho, L., Marcondes, M., 2010. Ocorrência de Leishmaniose em gatos de área endêmica para leishmaniose visceral. *Braz. J. Res. Anim. Sci.*, v47, n3, 213-217.
- Criado-Fornelio, A., Gutierrez-Garcia, L., Rodriguez-Caabeiro, F., Reus-Garcia, E., Roldan-Soriano, M., Diaz-Sanchez, M., 2000. A parasitological survey of wild red foxes (*Vulpes vulpes*) from the province of Guadalajara, Spain. *Veterinary Parasitology*, 92, 245-251.
- da Silva, A.V., de Souza Candido, C.D., de Pita Pereira, D., Brazil, R.P., Carreira, J.C., 2008. The first record of American visceral leishmaniasis in domestic cats from Rio de Janeiro, Brazil. *Acta tropica* 105, 92-94.
- da Silva, S.M., Rabelo, P.F., Gontijo Nde, F., Ribeiro, R.R., Melo, M.N., Ribeiro, V.M., Michalick, M.S., 2010. First report of infection of *Lutzomyia longipalpis* by *Leishmania*

- (*Leishmania*) *infantum* from a naturally infected cat of Brazil. Veterinary Parasitology 174, 150-154.
- Dahroug, M.A.A., Almeida, A.B.P.F., Sousa, V.R.F., Dutra, V., Guimarães, L.D., Soares, C.E., Nakazato, L., de Souza, R.L., 2011. The first case report of *Leishmania (leishmania) chagasi* in *Panthera leo* in Brazil. Asian Pacific Journal of Tropical Biomedicine 1, 249-250.
- de Moraes, R.C., Gonçalves Sda, C., Costa, P.L., da Silva, K.G., da Silva, F.J., Silva, R.P., de Brito, M.E., Brandão-Filho, S.P., Dantas-Torres, F., de Paiva-Cavalcanti, M., 2013. Detection of *Leishmania infantum* in animals and their ectoparasites by conventional PCR and real time PCR. Experimental & applied acarology 59, 473-481.
- Decreto-Lei 314/ 2013 de 17 Dezembro. Diário da República Portuguesa, n.º 290 - I Série. Ministério da Agricultura, Desenvolvimento Rural e Pescas.
- Diakou, A., Papadopoulos, E., Lazarides, K., 2009. Specific anti-*Leishmania* spp. antibodies in stray cats in Greece. Journal of feline medicine and surgery 11, 728-730.
- Fonseca, I. M. P., Brito, M. T., 2008. diagnóstico. In: Leishmaniose Canina. Merial, Lisboa, pp.83-91.
- Gibson-Corley, K.N., Hostetter, J.M., Hostetter, S.J., Mullin, K., Ramer-Tait, A.E., Boggiatto, P.M., Petersen, C.A., 2008. Disseminated *Leishmania infantum* infection in two sibling foxhounds due to possible vertical transmission. The Canadian veterinary journal. La revue veterinaire canadienne 49, 1005-1008.
- Gradoni, L., 2013. Epidemiological surveillance of leishmaniasis in the European Union: operational and research challenges. Eurosurveillance, vol18, 2-4.
- Gramiccia, M., Gradoni, L., 2005. The current status of zoonotic leishmaniasis and approaches to disease control. International journal for Parasitology 35, 1169-1180.
- Gramiccia, M., 2011. Recent advances in leishmaniosis in pet animals: epidemiology, diagnostics and anti-vectorial prophylaxis. Veterinary Parasitology 181, 23-30.
- Grevot, A., Jaussaud, H., Marty, P., Pratlong, F., Ozon, C., Haas, P., Breton, C., Bourdoiseau, G., 2005. Leishmaniose due to *Leishmania infantum* in a FIV and FeLV positive cat with squamous cell carcinoma diagnosed with histological, serological and isoenzymatic methods. Parasite Journal, 12, 271-275.

- Hatam, G., Adnani, S., Asgari, Q., Fallah, E., Motazedian, M., Sarkari, B., 2010. First report of natural infection in cats with *Leishmania infantum* in Iran. Vector Borne Zoonotic Disease, 10(3), 313-316.
- Hervás, J., De Lara, F., Sánchez-Isarria, M., Pellicer, S., Carrasco, L., Castillo, J., Gómez-Villamandos, J., 1999. Two cases of feline visceral and cutaneous leishmaniosis in Spain, Journal of Feline Medicine and Surgery, 1, 101:105.
- Koehler, K., Stechele, M., Hetxel, U., Domingo, M., Schonian, G., Zahner, H., Bukhardt, E., 2002. Cutaneous leishmaniosis in a horse in southern Germany caused by *Leishmania infantum*. Veterinary Parasitology, 109, 9-17.
- Killick-Kendrick, R., 2002. The life-cycles of *Leishmania* in the sand fly and transmission of leishmaniasis by bite. Intervet: Proceedings of the 2nd canine leishmaniasis forum.
- Leiva, M., Lloret, A., Peña, T., Roura, X., 2005. Therapy of ocular leishmaniasis in a cat. Veterinary Ophthalmology, 8, 1, 71-75.
- Longoni, S., López-Céspedes, A., Sánchez-Moreno, M., Boliio-Gonzales, M., Sauri-Arceo, C., Rodríguez-Vivas, R., Marín, C., 2012. Detection of different *Leishmania* spp. and *Trypanosoma cruzi* antibodies in cats from the Yucatan Peninsula (Mexico) using an iron superoxide dismutase excreted as antigen. Comparative Immunology, Microbiology and Infectious Diseases, 35, 469-476.
- Maia, C., Nunes, M., Campino, L., 2008. Importance of cats in zoonotic leishmaniasis in Portugal. Vector borne and zoonotic diseases 8, 555-559.
- Maia, C., Afonso, M.O., Neto, L., Dionisio, L., Campino, L., 2009. Molecular detection of *Leishmania infantum* in naturally infected *Phlebotomus perniciosus* from Algarve region, Portugal. Journal of vector borne diseases 46, 268-272.
- Maia, C., Gomes, J., Cristovao, J., Nunes, M., Martins, A., Rebelo, E., Campino, L., 2010. Feline *Leishmania* infection in a canine leishmaniasis endemic region, Portugal. Veterinary Parasitology 174, 336-340.
- Maia, C., Campino, L., 2011. Can domestic cats be considered reservoir hosts of zoonotic leishmaniasis? Trends in Parasitology 27, 341-344.

- Marcos, R., Santos, M., Malhao, F., Pereira, R., Fernandes, A.C., Montenegro, L., Roccabianca, P., 2009. Pancytopenia in a cat with visceral leishmaniasis. *Veterinary clinical pathology / American Society for Veterinary Clinical Pathology* 38, 201-205.
- Maroli, M., Pennisi, M.G., Di Muccio, T., Khoury, C., Gradoni, L., Gramiccia, M., 2007. Infection of sandflies by a cat naturally infected with *Leishmania infantum*. *Veterinary Parasitology* 145, 357-360.
- Martin-Sanchez, J., Acedo, C., Munoz-Perez, M., Pesson, B., Marchal, O., Morillas-Marquez, F., 2007. Infection by *Leishmania infantum* in cats: epidemiological study in Spain. *Veterinary Parasitology* 145, 267-273.
- Moraes-Silva, E., Antunes, F., Rodrigues, M., Julião, F., Dias-Lima, A., Lemos-de-Sousa, F., Alcantara, A., Reis, E., Nakatani, M., Badaaró, R., Reis, M., Pontes-de-Carvalho, L., Franke, C., 2006. Domestic swine in a visceral leishmaniasis endemic area produce antibodies against multiple *Leishmania infantum* antigens but apparently resist to *L. infantum*. *Acta Tropica*, 98, 176-182.
- Nasereddin, A., Salant, H., Abdeen, Z., 2008. Feline leishmaniasis in Jerusalem: serological investigation. *Veterinary Parasitology* 158, 364-369.
- Navarro, J.A., Sanchez, J., Penafiel-Verdu, C., Buendia, A.J., Altimira, J., Vilafranca, M., 2010. Histopathological lesions in 15 cats with leishmaniosis. *Journal of comparative pathology* 143, 297-302.
- Otranto, D., Dantas-Torres, F., de Caprariis, D., Di Paola, G., Tarallo, V.D., Latrofa, M.S., Lia, R.P., Annoscia, G., Breitshwerdt, E.B., Cantacessi, C., Capelli, G., Stanneck, D., 2013. Prevention of canine leishmaniosis in a hyper-endemic area using a combination of 10% imidacloprid/4.5% flumethrin. *PloS one* 8, e56374.
- Ozon, C., Marty, P., Pratlong, F., Breton, C., Blein, M., Lelièvre, A., Haas, P., 1998. Disseminated feline leishmaniosis due to *Leishmania infantum* in Southern France. *Veterinary Parasitology* 75, 273-277.
- Pennisi, G., 2002 A high prevalence of feline leishmaniasis in southern Italy. Intervet Precedings of the 2nd International canine leishmaniasis forum.
- Pennisi, M., Hartmann, K., Lloret, M., Addie, D., Belák, S., Boucraut-Baralon, C., Egberink, H., Frymus, T., Gruffyd-Jones, T., Hosie, M., Lutz, H., Marsilio, F., Mostl, K., Radford, A.,

- Thiry, E., Truyen, U., Horzinek, M., 2013. Leishmaniosis in cats: ABCD guidelines on prevention and management. *Journal of Feline Medicine and Surgery*, vol13, 638-642.
- Petersen, C.A., 2009. Leishmaniasis, an emerging disease found in companion animals in the United States. *Topics in companion animal medicine* 24, 182-188.
- Pocholle, E., Reyes-Gomez, E., Giacomo, A., Delaunay, P., Hasseine, L., Marty, P., 2012. Un cas de leishmaniose féline disséminée dans le sud de la France. *Parasite* 19, 77-80.
- Poli, A., Abramo, F., Barsotti, P., Leva, S., Gramiccia, M., Ludovisi, A., Mancianti, F., 2002. Feline leishmaniosis due to *Leishmania infantum* in Italy. *Veterinary Parasitology* 106, 181-191.
- Pratlong, F., Rioux, J.A., Marty, P., Faraut-Gambarelli, F., Dereure, J., Lanotte, G., Dedet, J.P., 2004. Isoenzymatic analysis of 712 strains of *Leishmania infantum* in the south of France and relationship of enzymatic polymorphism to clinical and epidemiological features. *Journal of clinical microbiology* 42, 4077-4082.
- Quinnell, R.J., Courtenay, O., 2009. Transmission, reservoir hosts and control of zoonotic visceral leishmaniasis. *Parasitology* 136, 1915-1934.
- Ready, P.D., 2010. Leishmaniasis emergence in Europe. *Euro surveillance : bulletin Européen sur les maladies transmissibles: European communicable disease bulletin* 15, 19505.
- Rolão, N., Martins, M.J., João, A., Campino, L., 2005. Equine infection with *Leishmania* in Portugal. *Parasite* 12, 183-186.
- Rüfenacht, S., Sager, h., Müller, N., Schaerer, V., Heier, A., Welle, M., Roosie, P.J., 2005. Two cases of feline leishmaniasis in Switzerland. *Veterinary Record* 165 (17), 542-525.
- Ruiz-Fons, F., 2013. *Leishmania infantum* in free-ranging hares, Spain, 2004-2010. *Eurosurveillance*, 18, 35-39.
- Santos-Gomes, G. M., Rodrigues, O. R., Marques, C., 2008. Resposta imunológica. In: *Leishmaniose Canina*. Merial, Lisboa, pp.69-80.
- Sastre, N., Francino, O., Ramirez, O., Ensenat, C., Sanchez, A., Altet, L., 2008. Detection of *Leishmania infantum* in captive wolves from Southwestern Europe. *Veterinary Parasitology* 158, 117-120.
- Savani, E.S., de Oliveira Camargo, M.C., de Carvalho, M.R., Zampieri, R.A., dos Santos, M.G., D'Auria, S.R., Shaw, J.J., Floeter-Winter, L.M., 2004. The first record in the Americas of

- an autochthonous case of *Leishmania (Leishmania) infantum chagasi* in a domestic cat (*Felis catus*) from Cotia County, Sao Paulo State, Brazil. *Veterinary Parasitology* 120, 229-233.
- Savani, E.S., de Almeida, M.F., de Oliveira Camargo, M.C., D'Auria, S.R., Silva, M.M., de Oliveira, M.L., Sacramento, D., 2010. Detection of *Leishmania (Leishmania) amazonensis* and *Leishmania (Leishmania) infantum chagasi* in Brazilian bats. *Veterinary Parasitology* 168, 5-10.
- Saz, S., Esteve, L. O., Solano-Gallego, L., 2013. Patogénesis y respuesta inmunitaria. In:., Cardoso L., Esteve L. O., Corrales G. M., Fondati A., Giménez M. T., Repiso M. L., Freixa C. N., Dantas-Torres F., Otranto D., Pennisi M. G., L., Pennisi (Eds), *Leishmaniose: una revisión actualizada*, 1st Ed. Navarra: Servet editorial, pp.33-35.
- Silva, F.L., Oliveira, R.G., Silva, T.M., Xavier, M.N., Nascimento, E.F., Santos, R.L., 2009. Venereal transmission of canine visceral leishmaniasis. *Veterinary Parasitology* 160, 55-59.
- Simoes-Mattos, L., Mattos, M.R., Teixeira, M.J., Oliveira-Lima, J.W., Bevilaqua, C.M., Prata-Junior, R.C., Holanda, C.M., Rondon, F.C., Bastos, K.M., Coelho, Z.C., Coelho, I.C., Barral, A., Pompeu, M.M., 2005. The susceptibility of domestic cats (*Felis catus*) to experimental infection with *Leishmania braziliensis*. *Veterinary Parasitology* 127, 199-208.
- Simões-Mattos, L., Bevilaqua, C., Maattos, M., Pompeu, M., 2004. Feline Leishmaniasis: uncommon or unknown? *Revista Portuguesa de Ciências Veterinárias*, 99 (550), 79-87.
- Sobrinho, L.S., Rossi, C.N., Vides, J.P., Braga, E.T., Gomes, A.A., de Lima, V.M., Perri, S.H., Generoso, D., Langoni, H., Leutenegger, C., Biondo, A.W., Laurenti, M.D., Marcondes, M., 2012. Coinfection of *Leishmania chagasi* with *Toxoplasma gondii*, Feline Immunodeficiency Virus (FIV) and Feline Leukemia Virus (FeLV) in cats from an endemic area of zoonotic visceral leishmaniasis. *Veterinary Parasitology* 187, 302-306.
- Solano-Gallego, L., Llull, J., Ramos, G., Riera, C., Arboix, M., Alberola, J., Ferrer, L., 2000. The Ibizaian hound presents a predominantly cellular immune response against natural *Leishmania* infection. *Veterinary Parasitology*, 90, 37-45.

- Solano-Gallego, L., Baneth, G., 2006. Feline Leishmaniasis. In: Infectious Diseases of the Dog and Cat, Elsevier, St. Louis, pp. 748-749.
- Solano-Gallego, L., Rodriguez-Cortes, A., Iniesta, L., Quintana, J., Pastor, J., Espada, Y., Portus, M., Alberola, J., 2007. Cross-sectional serosurvey of feline leishmaniasis in ecoregions around the Northwestern Mediterranean. The American journal of tropical medicine and hygiene 76, 676-680.
- Solano-Gallego, Cardoso, L., 2013. Epidemiologia en Europa. In: Saz, S., Esteve, L. O. Esteve L. O., Corrales G. M., Fondati A., Giménez M. T., Repiso M. L., Freixa C. N., Dantas-Torres F., Otranto D., Pennisi M. G., L., Pennisi (Eds), Leishmaniose: una revisión actualizada, 1st Ed. Navarra: Servet editorial, pp.17-30.
- Spada, E., Proverbio, D., Migliazzo, A., Della Pepa, A., Perego, R., Bagnagatti De Giorgi, G., 2013. Serological and Molecular Evaluation of *Leishmania infantum* Infection in Stray Cats in a Nonendemic Area in Northern Italy. ISRN Parasitology 2013, 1-6.
- Stanneck, D., Rass, J., Radeloff, I., Kruehwagen, E., Le Sueur, C., Hellmann, K., Krieger, K., 2012a. Evaluation of the long-term efficacy and safety of an imidacloprid 10%/flumethrin 4.5% polymer matrix collar (Seresto®) in dogs and cats naturally infested with fleas and/or ticks in multicentre clinical field studies in Europe. Parasites & vectors 5, 66.
- Stanneck, D., Kruehwagen, E., Fourle, J., Horak, I., Davis, W., Krieger, K., 2012b. Efficacy of an imidacloprid/ flumethrin collar against fleas and ticks on cats. Parasites & Vectors, 5, 82.
- Tomás, A. M., Romão, S. F., 2008. Patogenia e lesões da leishmaniose canina . In: Leishmaniose Canina. Merial, Lisboa, pp.53-67.
- Trainor, K.E., Porter, B.F., Logan, K.S., Hoffman, R.J., Snowden, K.F., 2010. Eight cases of feline cutaneous leishmaniasis in Texas. Veterinary pathology 47, 1076-1081.
- Velázquez, A., Medina, M., Pedrozo, R., Miret, J., Coiro C., Generoso, G., Kikuti, M., da Silva, R., Langoni, L., 2011. Prevalencia de anticuerpos anti-*Leishmania infantum* bpor inmunofluorescencia indirecta (IFI) y estudio de factores de riesgo en gatos domésticos en el Paraguay, Veterinaria e Zootecnia, 18, 284-296.
- Vides, J.P., Schwardt, T.F., Sobrinho, L.S., Marinho, M., Laurenti, M.D., Biondo, A.W., Leutenegger, C., Marcondes, M., 2011. *Leishmania chagasi* infection in cats with

- dermatologic lesions from an endemic area of visceral leishmaniosis in Brazil. *Veterinary Parasitology* 178, 22-28.
- Vilhena, H., Martinez-Diaz, V.L., Cardoso, L., Vieira, L., Altet, L., Francino, O., Pastor, J., Silvestre-Ferreira, A.C., 2013. Feline vector-borne pathogens in the north and centre of Portugal. *Parasites & vectors* 6, 99.
- Vita, S., Santori, D., Aguzzi, I., Petrotta, E., Luciani, A., 2005. Feline leishmaniasis and ehrlichiosis: serological investigation in Abruzzo region. *Veterinary research communications* 29 Suppl 2, 319-321.

Annex

Guide for authors of the journal Veterinary Parasitology (retrieved from:

<http://www.elsevier.com/journals/veterinary-parasitology/0304-4017/guide-for-authors>):

Veterinary Parasitology

Types of contributions

1. Original research papers (Regular Papers)
2. Review articles
3. Rapid Communications
4. Short Communications
5. Letters to the Editor
6. Book Reviews

Original research papers should report the results of original research. The material should not have been previously published elsewhere, except in a preliminary form.

Review articles should cover subjects falling within the scope of the journal which are of active current interest. They may be submitted or invited.

Rapid Communications should contain information of high 'news'/scientific value worthy of very rapid publication. Rapid Communications should be submitted to the journal as such (i.e. clearly labelled as a RC) and should, in general, not exceed 2000 words in length. Upon receipt, they will be subject to rapid assessment and if accepted, published with priority.

Short Communications should consist of original observations or new methods within the scope of the journal. Reports of observations previously published from different geographical areas may be accepted only if considered sufficiently unusual or noteworthy. The Communications should be concise with the minimum of references, and cover no more than four pages of the journal; they need not be formally structured as are full papers, but should give sufficient methods and data necessary for their comprehension.

Letters to the Editor offering comment or useful critique on material published in the journal are welcomed. The decision to publish submitted letters rests purely with the Editors-in-Chief. It is hoped that the publication of such letters will permit an exchange of views which will be of benefit to both the journal and its readers.

Book Reviews will be included in the journal on a range of relevant books which are not more than 2 years old and were written in English.

Book reviews will be solicited by the Book Review Editor. Unsolicited reviews will not usually be accepted, but suggestions for appropriate books for review may be sent to the Book Review Editor:

Dr W. Pomroy

Institute of Veterinary, Animal and Biomedical Sciences

Massey University

Private Bag 11 222

Palmerston North 4442

New Zealand

w.pomroy@massey.ac.nz

Submission of manuscripts

Submission to *Veterinary Parasitology* now proceeds online via Elsevier Editorial System - <http://ees.elsevier.com/vetpar>. Authors will be guided step-by-step through uploading files directly from their computers. Authors should select a set of classifications for their papers from a given list, as well as a category designation (Original Research Paper, Short Communication, and so on). Electronic PDF proofs will be automatically generated from uploaded files, and used for subsequent reviewing.

A cover letter is required for each new submission. It should address the novelty and significance of the work and how it fits within the defined scope of *Veterinary Parasitology*. Essential information, issues of concern or potential problems, (such as other publications or submissions containing similar information) should be identified in the cover letter. Authors who submit papers based on local data/surveys will need to indicate why their paper is relevant to a broader readership.

Authors are invited to suggest the names of up to 5 referees (with email addresses) whom they feel are qualified to evaluate their submission. Submission of such names does not, however, imply that they will definitely be used as referees.

Authors should send queries concerning the submission process or journal procedures to AuthorSupport@elsevier.com. Authors can check the status of their manuscript within the review procedure using Elsevier Editorial System.

Authors submitting hard copy papers will be asked to resubmit using Elsevier Editorial System.

Submission of an article is understood to imply that the article is original and is not being considered for publication elsewhere. Submission also implies that all authors have approved the paper for release and are in agreement with its content. Upon acceptance of the article by the journal, the author(s) will be asked to transfer the copyright of the article to the Publisher. This transfer will ensure the widest possible dissemination of information.

All authors should have made substantial contributions to all of the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted.

Acknowledgements

All contributors who do not meet the criteria for authorship as defined above should be listed in an acknowledgements section. Examples of those who might be acknowledged include a person who provided purely technical help, writing assistance, or a department chair who provided only general support. Authors should disclose whether they had any writing assistance and identify the entity that paid for this assistance.

Conflict of interest

At the end of the text, under a subheading "Conflict of interest statement" all authors must disclose any financial and personal relationships with other people or organisations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

Role of the funding source

All sources of funding should be declared as an acknowledgement at the end of the text. Authors should declare the role of study sponsors, if any, in the study design, in the collection, analysis and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication. If the study sponsors had no such involvement, the authors should so state.

Open Access

This journal offers authors two choices to publish their research;

1. Open Access

- Articles are freely available to both subscribers and the wider public with permitted reuse
- An Open Access publication fee is payable by authors or their research funder

2. Subscription

- Articles are made available to subscribers as well as developing countries and patient groups through our access programs (<http://www.elsevier.com/access>)
- No Open Access publication fee

All articles published Open Access will be immediately and permanently free for everyone to read and download. Permitted reuse is defined by your choice of one of the following Creative Commons user licenses:

Creative Commons Attribution-Non Commercial-ShareAlike (CC BY-NC-SA): for non-commercial purposes, lets others distribute and copy the article, to create extracts, abstracts and other revised versions, adaptations or derivative works of or from an article (such as a translation), to include in a collective work (such as an anthology), to text and data mine the article, as long as they credit the author(s), do not represent the author as endorsing their adaptation of the article, do not modify the article in such a way as to damage the author's honor or reputation, and license their new adaptations or creations under identical terms (CC BY NC SA).

Creative Commons Attribution-NonCommercial-NoDerivs (CC-BY-NC-ND): for non-commercial purposes, lets others distribute and copy the article, and to include in a collective work (such as an anthology), as long as they credit the author(s) and provided they do not alter or modify the article.

Creative Commons Attribution (CC-BY): available only for authors funded by organizations with which we have established an agreement with. For a full list please see www.elsevier.com/fundingbodies

Elsevier has established agreements with funding bodies. This ensures authors can comply with funding body Open Access requirements, including specific user licenses, such as CC-BY. Some authors may also be reimbursed for associated publication fees. www.elsevier.com/fundingbodies

To provide Open Access, this journal has a publication fee which needs to be met by the authors or their research funders for each article published Open Access. Your publication choice will have no effect on the peer review process or acceptance of submitted articles.

The Open Access publication fee for this journal is \$USD 2,500, excluding taxes.

Learn more about Elsevier's pricing policy

www.elsevier.com/openaccesspricing

Ethics

Circumstances relating to animal experimentation must meet the International Guiding Principles for Biomedical Research Involving Animals as issued by the Council for the International Organizations of Medical Sciences. They are obtainable from: Executive Secretary C.I.O.M.S., c/o WHO, Via Appia, CH-1211 Geneva 27, Switzerland, or at the following URL: http://www.cioms.ch/publications/guidelines/1985_texts_of_guidelines.htm. Unnecessary cruelty in animal experimentation is not acceptable to the Editors of *Veterinary Parasitology*.

Preparation of manuscripts

1. Manuscripts should be written in English. Authors whose native language is not English are strongly advised to have their manuscripts checked by an English-speaking colleague prior to submission.

Language Editing: [Elsevier's Authors Home](http://www.elsevier.com/authors/home) provides details of some companies who can provide English language and copyediting services to authors who need assistance *before* they submit their article or *before* it is accepted for publication. Authors should contact these services directly. Authors should also be aware that *The Lucidus Consultancy* edit@lucidusconsultancy.com offers a bespoke service to putative

contributors to Veterinary Parasitology who need to arrange language improvement for their manuscripts. For more information about language editing services, please email authorsupport@elsevier.com.

Please note that Elsevier neither endorses nor takes responsibility for any products, goods or services offered by outside vendors through our services or in any advertising. For more information please refer to our terms & conditions <http://www.elsevier.com/termsandconditions>.

2. Manuscripts should have **numbered lines**, with wide margins and **double spacing** throughout, i.e. also for abstracts, footnotes and references. **Every page of the manuscript, including the title page, references, tables, etc., should be numbered.** However, in the text no reference should be made to page numbers; if necessary one may refer to sections. Avoid excessive usage of italics to emphasize part of the text.

3. Manuscripts in general should be organized in the following order:

Title (should be clear, descriptive and not too long)

Name(s) of author(s)

Complete postal address(es) of affiliations

Full telephone, Fax No. and e-mail address of the corresponding author

Present address(es) of author(s) if applicable

Complete correspondence address including e-mail address to which the proofs should be sent

Abstract

Keywords (indexing terms), normally 3-6 items. Please refer to last index (Vol. 100/3-4).

Introduction

Material studied, area descriptions, methods, techniques

Results

Discussion

Conclusion

Acknowledgments and any additional information concerning research grants, etc.

References

Tables

Figure captions

Tables (separate file(s))

Figures (separate file(s)).

4. Titles and subtitles should not be run within the text. They should be typed on a separate line, without indentation. Use lower-case letter type.

5. SI units should be used.

6. Elsevier reserves the privilege of returning to the author for revision accepted manuscripts and illustrations which are not in the proper form given in this guide.

Abstracts

The abstract should be clear, descriptive and not longer than 400 words.

Tables

1. Authors should take notice of the limitations set by the size and lay-out of the journal. Large tables should be avoided. Reversing columns and rows will often reduce the dimensions of a table.

2. If many data are to be presented, an attempt should be made to divide them over two or more tables.

3. Tables should be numbered according to their sequence in the text. The text should include references to all tables.

4. Each table should occupy a separate page of the manuscript. Tables should never be included in the text.

5. Each table should have a brief and self-explanatory title.

6. Column headings should be brief, but sufficiently explanatory. Standard abbreviations of units of measurement should be added between parentheses.

7. Vertical lines should not be used to separate columns. Leave some extra space between the columns instead.

8. Any explanation essential to the understanding of the table should be given as a footnote at the bottom of the table.

Illustrations

1. All illustrations (line drawings and photographs) should be submitted as separate files, preferably in TIFF or EPS format.

2. Illustrations should be numbered according to their sequence in the text. References should be made in the text to each illustration.

3. Illustrations should be designed with the format of the page of the journal in mind. Illustrations should be of such a size as to allow a reduction of 50%.

4. Lettering should be big enough to allow a reduction of 50% without becoming illegible. Any lettering should be in English. Use the same kind of lettering throughout and follow the style of the journal.

5. If a scale should be given, use bar scales on all illustrations instead of numerical scales that must be changed with reduction.

6. Each illustration should have a caption. The captions to all illustrations should be typed on a separate sheet of the manuscript.

7. Explanations should be given in the figure legend(s). Drawn text in the illustrations should be kept to a minimum.

8. Photographs are only acceptable if they have good contrast and intensity.

9. If you submit usable colour figures, Elsevier would ensure that these figures appeared free-of-charge in colour in the electronic version of your accepted paper, regardless of whether or not these illustrations are reproduced in colour in the printed version. Colour illustrations can only be included in print if the additional cost of reproduction is contributed by the author: you would receive information regarding the costs from Elsevier after receipt of your accepted article.

Please note that because of technical complications which may arise by converting colour figures to 'grey scale' (for the printed version, should you not opt for colour in print), you should submit in addition usable black and white figures corresponding to all colour illustrations.

10. Advice on the preparation of illustrations can be found at the following URL: <http://www.elsevier.com/artworkinstructions>

AudioSlides

The journal encourages authors to create an AudioSlides presentation with their published article. AudioSlides are brief, webinar-style presentations that are shown next to the online article on ScienceDirect. This gives authors the opportunity to summarize their research in their own words and to help readers understand what the paper is about. More information and examples are available at <http://www.elsevier.com/audioslides>. Authors of this journal will automatically receive an invitation e-mail to create an AudioSlides presentation after acceptance of their paper. **Preparation of supplementary data**

Elsevier now accepts electronic supplementary material to support and enhance your scientific research. Supplementary files offer the author additional possibilities to publish supporting applications, movies, animation sequences, high-resolution images, background datasets, sound clips and more. Supplementary files supplied will be published free of charge online alongside the electronic version of your article in Elsevier web products, including ScienceDirect: <http://www.sciencedirect.com>. In order to ensure that your submitted material is directly usable, please ensure that data are provided in one of our recommended file formats. Authors should submit the material together with the article and supply a concise and descriptive caption for each file. For more detailed instructions please visit <http://www.elsevier.com/artworkinstructions>.

References

1. All publications cited in the text should be presented in a list of references following the text of the manuscript. The manuscript should be carefully checked to ensure that the spelling of author's names and dates are exactly the same in the text as in the reference list.

2. In the text refer to the author's name (without initial) and year of publication, followed – if necessary – by a short reference to appropriate pages. Examples: "Since Peterson (1988) has shown that..." "This is in agreement with results obtained later (Kramer, 1989, pp. 12–16)".

3. If reference is made in the text to a publication written by more than two authors the name of the first author should be used followed by "et al.". This indication, however, should never be used in the list of references. In this list names of first author and co-authors should be mentioned.

4. References cited together in the text should be arranged chronologically. The list of references should be arranged alphabetically on author's names, and chronologically per author. If an author's name in the list is also mentioned with co-authors the following order should be used: publications of the single author, arranged according to publication dates – publications of the same author with one co-author – publications of the author with more than one co-author. Publications by the same author(s) in the same year should be listed as 1974a, 1974b, etc.

5. Use the following system for arranging your references:

a. For periodicals

Lanusse, C.E., Prichard, R.K., 1993. Relationship between pharmacological properties and clinical efficacy of ruminant anthelmintics. *Vet. Parasitol.* 49, 123–158.

b. For edited symposia, special issues, etc., published in a periodical

Weatherley, A.J., Hong, C., Harris, T.J., Smith, D.G., Hammet, N.C., 1993. Persistent efficacy of doramectin against experimental nematode infections in calves. In: Vercruysse, J. (Ed.), *Doramectin – a novel avermectin*. *Vet. Parasitol.* 49, 45–50.

c. For books

Blaha, T. (Ed.), 1989. *Applied Veterinary Epidemiology*. Elsevier, Amsterdam, 344 pp.

d. For multi-author books

Wilson, M.B., Nakane, P.K., 1978. Recent developments in the periodate method of conjugating horseradish peroxidase (HRPO) to antibodies. In: Knapp, W., Holubar, K., Wick, G. (Eds.), *Immunofluorescence and Related Staining Techniques*. North Holland, Amsterdam, pp. 215–224.

6. Abbreviate the titles of periodicals mentioned in the list of references in accordance with BIOSIS Serial Sources, published annually by BIOSIS. The correct abbreviation for this journal is *Vet. Parasitol.*

7. In the case of publications in any language other than English, the original title is to be retained. However, the titles of publications in non-Latin alphabets should be transliterated, and a notation such as "(in Russian)" or "(in Greek, with English abstract)" should be added.

8. Work accepted for publication but not yet published should be referred to as "in press".

9. References concerning unpublished data and "personal communications" should not be cited in the reference list but may be mentioned in the text.

10. Web references may be given. As a minimum, the full URL is necessary. Any further information, such as Author names, dates, reference to a source publication and so on, should also be given.

11. Articles available online but without volume and page numbers may be referred to by means of their Digital Object identifier (DOI) code.

Formulae

1. Give the meaning of all symbols immediately after the equation in which they are first used.
2. For simple fractions use the solidus (/) instead of a horizontal line.
3. Equations should be numbered serially at the right-hand side in parentheses. In general only equations explicitly referred to in the text need be numbered.
4. The use of fractional powers instead of root signs is recommended. Powers of e are often more conveniently denoted by exp.
5. In chemical formulae, valence of ions should be given as, e.g. Ca^{2+} , not as Ca^{++} .
6. Isotope numbers should precede the symbols e.g. ^{18}O .
7. The repeated use of chemical formulae in the text is to be avoided where reasonably possible; instead, the name of the compound should be given in full. Exceptions may be made in the case of a very long name occurring very frequently or in the case of a compound being described as the end product of a gravimetric determination (e.g. phosphate as P_2O_5).

Footnotes

1. Footnotes should only be used if absolutely essential. In most cases it should be possible to incorporate the information into the normal text.
2. If used, they should be numbered in the text, indicated by superscript numbers, and kept as short as possible.

Nomenclature

1. Authors and editors are, by general agreement, obliged to accept the rules governing biological nomenclature, as laid down in the *International Code of Botanical Nomenclature*, the *International Code of Nomenclature of Bacteria*, and the *International Code of Zoological Nomenclature*.
2. All botica (crops, plants, insects, birds, mammals, etc.) should be identified by their scientific names when the English term is first used, with the exception of common domestic animals.
3. All biocides and other organic compounds must be identified by their Geneva names when first used in the text. Active ingredients of all formulations should be likewise identified.
4. For chemical nomenclature, the conventions of the *International Union of Pure and Applied Chemistry* and the official recommendations of the *IUPAC-IUB Combined Commission on Biochemical Nomenclature* should be followed.
5. For the denomination of parasitic diseases or infections, authors are advised to consult the Standardized Nomenclature of Animal Parasitic Diseases (SNOAPAD) published in 1988 in *Veterinary Parasitology* (Kassai, T. et al., 1988. *Vet. Parasitol.* 29, 299-326).

Submission of sequence data to databases

New nucleotide or amino acid sequence data must be deposited in publicly accessible databases, such as GenBank™, EMBL or DDJB, and an accession number obtained and submitted to the Publisher (at the latest) together with the final, revised manuscript. The accession number should appear in a separate paragraph in the Materials and Methods section (example: Nucleotide sequence data reported in this paper are available in the GenBank™, EMBL and DDBJ databases under the accession numbers: XXXX, XXXX). In order for automatic links to be made between papers and databases, authors should type the accession number in bold, underlined text. Letters in the accession number should always be capitalised. When published they will appear in normal type.

Elsevier encourages authors to connect articles with external databases, giving their readers one-click access to relevant databases that help to build a better understanding of the described research. Please refer to <http://www.elsevier.com/databaselinking> for more information and a full list of supported databases.

Copyright

If excerpts from other copyrighted works are included, the Author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by Authors in these cases: contact Elsevier's Rights Department, Oxford, UK: phone (+1) 215 239 3804 or +44(0)1865 843830, fax +44(0)1865 853333, e-mail healthpermissions@elsevier.com. Requests may also be completed online via the Elsevier homepage <http://www.elsevier.com/permissions>.

Material in unpublished letters and manuscripts is also protected and must not be published unless permission has been obtained.

Authors Rights

As an author you (or your employer or institution) may do the following:

- make copies (print or electronic) of the article for your own personal use, including for your own classroom teaching use
- make copies and distribute such copies (including through e-mail) of the article to research colleagues, for the personal use by such colleagues (but not commercially or systematically, e.g., via an e-mail list or server)
- post a pre-print version of the article on Internet websites including electronic pre-print servers, and to retain indefinitely such version on such servers or sites
- post a revised personal version of the final text of the article (to reflect changes made in the peer review and editing process) on your personal or institutional website or server, with a link to the journal homepage

- (on [elsevier.com](http://www.elsevier.com))
- present the article at a meeting or conference and to distribute copies of the article to the delegates attending such a meeting
 - for your employer, if the article is a 'work for hire', made within the scope of your employment, your employer may use all or part of the information in the article for other intra-company use (e.g., training)
 - retain patent and trademark rights and rights to any processes or procedure described in the article
 - include the article in full or in part in a thesis or dissertation (provided that this is not to be published commercially)
 - use the article or any part thereof in a printed compilation of your works, such as collected writings or lecture notes (subsequent to publication of your article in the journal)
 - prepare other derivative works, to extend the article into book-length form, or to otherwise re-use portions or excerpts in other works, with full acknowledgement of its original publication in the journal

Funding body agreements and policies

Elsevier has established agreements and developed policies to allow authors who publish in Elsevier journals to comply with potential manuscript archiving requirements as specified as conditions of their grant awards. To learn more about existing agreements and policies please visit <http://www.elsevier.com/fundingbodies>).

Proofs

One set of page proofs in PDF format will be sent by e-mail to the corresponding author (if we do not have an e-mail address then paper proofs will be sent by post). Elsevier now sends PDF proofs which can be annotated; for this you will need to download Adobe Reader version 7 available free from <http://www.adobe.com/products/acrobat/readstep2.html>. Instructions on how to annotate PDF files will accompany the proofs. The exact system requirements are given at the Adobe site: <http://www.adobe.com/products/acrobat/acrrsystemreqs.html#70win>. If you do not wish to use the PDF annotations function, you may list the corrections (including replies to the Query Form) and return to Elsevier in an e-mail. Please list your corrections quoting line number. If, for any reason, this is not possible, then mark the corrections and any other comments (including replies to the Query Form) on a printout of your proof and return by fax, or scan the pages and e-mail, or by post. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. We will do everything possible to get your article published quickly and accurately. Therefore, it is important to ensure that all of your corrections are sent back to us in one communication: please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility.

Authors can also keep a track of the progress of their accepted article, and set up e-mail alerts informing them of changes to their manuscript's status, by using the "Track your accepted article" option on the journal's homepage <http://www.elsevier.com/locate/vetpar>. For privacy, information on each article is password-protected. The author should key in the "Our Reference" code (which is in the letter of acknowledgement sent by the Publisher on receipt of the accepted article) and the name of the corresponding author.

Offprints

The corresponding author will, at no cost, be provided with a PDF file of the article via e-mail. The PDF file is a watermarked version of the published article and includes a cover sheet with the journal cover image and a disclaimer outlining the terms and conditions of use.

Veterinary Parasitology has no page charges